📄 1 – Introduction & Importance of Microscopy 🔬

🔹 What is Microscopy?

- Science of using microscopes to **view objects invisible to the naked eye**.
- Key tool in **biology, medicine, nanotechnology, forensics, and industry**.

🔹 Why Microscopy is Important?

1. **Biology** → study of cells, tissues, microorganisms.
2. **Medicine** → diagnosis (e.g., tuberculosis bacteria, blood smears, cancer histology).
3. **Research** → ultrastructure of organelles (mitochondria, chloroplast).
4. **Industry** → food safety, textiles, materials science.
5. **Environmental studies** → microbes in soil, water quality.

🔹 Core Principles in Microscopy

- **Magnification** → makes object appear larger.
- **Resolution** → ability to distinguish two close points separately.
- **Contrast** → difference between specimen & background.

🔹 Key Formula

- **Resolving Power (d) = λ / (2 × NA)**
    - λ = wavelength of light/electrons.
    - NA = numerical aperture of lens.
- Smaller **d** = higher resolution.

🔹 Mnemonics 💡

- **Microscopy Uses → "BRIME"**
    - **B**iology
    - **R**esearch
    - **I**ndustry
    - **M**edicine
    - **E**nvironment

🔹 Exam Capsule 🎯

- Microscopy = visualization of objects < 0.1 mm.
- Depends on **magnification, resolution, contrast**.
- Resolving power (Abbe's law) is the **ultimate limit** of microscopy.
- Applied in **biology, pathology, nanotech, environmental science**.

📄 2 – Principles of Microscopy 🔬📐

🔹 1. Magnification

- Process of **enlarging the image** of a specimen.
- Achieved by **objective × eyepiece lenses**.
- Example: 40× (objective) × 10× (eyepiece) = **400× total magnification**.

🔹 2. Resolution (Resolving Power)

- Ability to distinguish **two close points separately**.
- Higher resolution = clearer detail.
- Governed by **Abbe's Law**:
  - **$d = \lambda / (2 \times NA)$**
  - λ = wavelength of illumination
  - NA = Numerical Aperture

🔹 3. Numerical Aperture (NA)

- Lens property defining its light-gathering ability.
- **$NA = n \times \sin\theta$**
  - n = refractive index of medium between lens & specimen (air = 1, oil = 1.5).
  - θ = half-angle of light cone entering lens.
- Higher NA = better resolution.

🔹 4. Contrast

- Difference in light intensity between specimen and background.
- Can be improved by stains (light microscopy) or phase contrast techniques.

🔹 5. Limits of Light Microscopy

- Visible light λ = 400–700 nm → **limit of resolution ~200 nm**.
- Cannot resolve viruses, ribosomes, macromolecules (need electron microscopy).

🔹 Mnemonics 💡

- **Principles → "MRCN"**
  - **M**agnification
  - **R**esolution
  - **C**ontrast
  - **N**umerical aperture

🔹 Exam Capsule 🎯

- **Magnification** enlarges, but **resolution** defines clarity.
- **Abbe's law** → smaller λ & larger NA = better resolution.
- **Oil immersion** increases NA (n = 1.5), hence resolution.
- Limit of resolution of light microscope = **~200 nm**.

📄 3 – Applications of Microscopy 🔬🌍

🔹 1. Biological Sciences

- Cell structure & organelles (nucleus, chloroplast, mitochondria).
- Microorganisms (bacteria, fungi, protozoa, algae).
- Plant anatomy & histology (tissues, meristems, vascular bundles).

🔹 2. Medical Sciences

- Clinical diagnosis (blood smears, malaria parasite, tuberculosis bacillus).
- Histopathology → study of cancerous & diseased tissues.
- Virology & microbiology (HIV, influenza, bacterial infections).

🔹 3. Agriculture & Botany

- Study of pollen grains & spores (palynology).
- Identification of plant pathogens.
- Observation of root nodules & mycorrhiza.

🔹 4. Industrial Applications

- Food industry → detection of contaminants, food adulteration.
- Textile industry → fiber quality checks.
- Paper & wood industry → fiber length, pulp structure.
- Forensics → hair, fibers, fingerprints, soil particles.

🔹 5. Environmental Studies

- Monitoring water quality → plankton & algal blooms.
- Soil microbial diversity.
- Pollution studies (toxic micro-particles, microplastics).

🔹 Mnemonics 💡

- **Applications → "BAMIE"**
  - **B**iology
  - **A**griculture
  - **M**edicine
  - **I**ndustry
  - **E**nvironment

🔹 Exam Capsule 🎯

- Microscopy = **core tool** in all sciences.
- Biology → organelles, microbes.
- Medicine → diagnostics, pathology.
- Agriculture → pollen, pathogens.
- Industry → fibers, quality control.
- Environment → soil, water microbes, pollutants.

📄 4 – Light Microscopy (Simple vs. Compound) 🔬💡

🔹 1. Simple Microscope

- Uses a **single convex lens**.
- Works like a magnifying glass.
- Magnification: up to **10–25×**.
- Example: Hand lens, dissection microscope.
- Limitation: **low resolution**.

🔹 2. Compound Microscope

- Uses **two sets of lenses**:
  - **Objective lens** (near specimen) → primary magnification.
  - **Eyepiece (ocular) lens** (near eye) → further magnification.
- Total magnification = **Objective × Eyepiece**.
- Magnification: up to **1000–1500×**.
- Resolution limit: ~ **200 nm** (due to visible light wavelength).

🔹 3. Parts of a Compound Microscope

- **Mechanical Parts**:
  - Base, arm, stage, clips, coarse & fine adjustment knobs.
- **Optical Parts**:
  - **Illumination system**: mirror/lamp + condenser + diaphragm.
  - **Objective lenses**: 4×, 10×, 40×, 100× (oil immersion).
  - **Eyepiece**: usually 5×–15×.
- **Oil Immersion Technique**: increases refractive index, reduces light scattering → better resolution.

🔹 4. Advantages of Light Microscopy

- Inexpensive, easy to use.
- Live cell observation possible.
- Good for teaching & basic research.

🔹 5. Limitations

- Limited resolution (~200 nm).
- Cannot visualize viruses, ribosomes, macromolecules.
- Requires staining for contrast.

🔹 **Mnemonics** 💡

- **Compound Microscope Lens Combo → "OE"**
  - **O**bjective
  - **E**yepiece

🔹 **Exam Capsule** 🎯

- **Simple microscope** → single lens, low magnification.
- **Compound microscope** → objective + eyepiece, up to 1500×.
- Limit of resolution = **200 nm**.
- Uses → cells, tissues, microorganisms.
- Oil immersion → improves resolution by increasing NA.

📄 5 – Specialized Optical Microscopes 🔬🔎

🔹 **1. Inverted Microscope**

- Light source & condenser **above stage**; objectives **below stage**.
- Ideal for **cell culture studies** → cells at bottom of culture flasks can be observed.
- Common in **tissue culture, embryology, live-cell imaging**.

🔹 **2. Stereoscopic (Dissecting) Microscope**

- Provides a **3D view** of specimen.
- Uses two separate optical paths.
- Low magnification (10×–80×).
- Applications: **dissections, entomology, fossils, surface study of organs**.

🔹 **3. Metallographic Microscope**

- Designed to examine **metals, alloys, polished surfaces**.

- Uses reflected light (not transmitted).
- Widely used in **material science, metallurgy, failure analysis**.

🔹 4. Reflecting Microscope

- Uses **mirrors instead of lenses** to avoid chromatic aberration.
- Employed in **astronomy & material sciences**.
- Rare in biology, but good for **specimens with strong light scattering**.

🔹 Mnemonics 💡

- **Special Microscopes → "ISMR"**
  - **I**nverted → cell culture.
  - **S**tereoscopic → 3D dissection.
  - **M**etallographic → metals.
  - **R**eflecting → mirror-based, special optics.

🔹 Exam Capsule 🎯

- **Inverted microscope** → best for live cell & tissue culture.
- **Stereoscopic microscope** → 3D low magnification view.
- **Metallographic microscope** → metals & alloys (reflected light).
- **Reflecting microscope** → mirrors, eliminates chromatic aberration.

📄 6 – Brightfield Microscope 🔬💡

🔹 Principle

- Light passes through specimen → specimen absorbs some light → contrast created between specimen & background.
- Image appears **dark against a bright background**.

◈ Components

- **Illumination system**: light source + condenser + diaphragm.
- **Objective lenses**: magnify specimen.
- **Eyepiece**: secondary magnification.
- **Stage & adjustment knobs**: positioning & focusing specimen.

◈ Applications

- Study of **stained specimens** (cells, tissues, microorganisms).
- **Histology** → animal & plant tissue sections.
- **Microbiology** → bacteria (after staining: Gram, acid-fast).
- Basic **botany & zoology practicals** in labs.

◈ Advantages

- Easy to operate, inexpensive.
- Suitable for **stained, fixed specimens**.
- Good resolution up to **200 nm**.

◈ Limitations

- Poor natural contrast (requires staining).
- Not suitable for live, transparent cells.
- Limited resolving power (cannot see viruses, macromolecules).

◈ Mnemonics 💡

- **Brightfield Image → "Dark on Bright"** = specimen dark, background bright.

◈ Exam Capsule 🎯

- **Brightfield microscope** = simplest & most widely used type.
- Works on principle of **light absorption by specimen**.
- Best for **stained, dead samples**.
- Resolution limit ~ **200 nm**.
- Not suitable for live cell observation.

📄 7 – Darkfield Microscope 🦠✨

🔹 Principle

- Uses a **special condenser** that blocks central light rays.
- Only **oblique rays** hit the specimen → scattered light enters objective.
- Image = **bright specimen on a dark background**.

🔹 Key Features

- **No staining required**.
- Increases contrast of **transparent, unstained specimens**.
- Can reveal structures invisible in brightfield.

🔹 Applications

- Observation of **living, unstained cells** (e.g., protozoa, spirochetes).
- **Medical microbiology** → Treponema pallidum (syphilis pathogen).
- **Aquatic biology** → motile plankton, small aquatic organisms.
- Useful for **thin bacterial structures** (flagella).

🔹 Advantages

- High contrast without staining.
- Visualizes **live, motile cells**.
- Reveals **fine structures** like spirochetes, flagella.

🔹 Limitations

- Not suitable for thick specimens (scatter too much light).
- Cannot provide detailed internal structures.
- Requires special condenser → more expensive.

🔹 Mnemonics 💡

- **Darkfield → "Bright Bugs in Dark"** (organism glows against black background).

🔹 Exam Capsule 🎯

- **Darkfield microscopy** → bright specimen, dark background.
- Works by **oblique scattered light**.
- Best for **live, unstained, transparent cells**.
- Medical use → **Treponema pallidum** (syphilis).
- Limitation → no internal details, not for thick samples.

📄 8 – Phase-Contrast Microscope 🎨🔬

🔹 Principle

- Transparent cells **do not absorb much light** → appear nearly invisible in brightfield.
- Phase-contrast converts **differences in refractive index** (light phase shifts) into **differences in brightness (contrast)**.
- Uses: **Annular diaphragm** + **Phase plate**.

🔹 Components

1. **Annular Diaphragm** → produces hollow cone of light.
2. **Phase Plate** → shifts light phase from specimen, creating constructive/destructive interference.
3. **Objective Lens** → collects refracted + direct light.

🔹 Image Produced

- Transparent specimen structures appear as **light & dark areas** without staining.
- Live cells can be observed in natural state.

🔹 Applications

- **Cell biology** → live cell observation (nucleus, mitochondria, vacuoles).
- **Microbiology** → bacteria, protozoa, fungal hyphae.
- **Cytology** → study of mitosis, meiosis, endocytosis, exocytosis.
- **Medical research** → sperm motility, tissue culture.

🔹 Advantages

- No staining → specimen remains alive.

- Reveals internal structures (nucleus, organelles).
- High contrast images of transparent cells.

🔹 Limitations

- Expensive (requires special optics).
- Not suitable for very thick specimens.
- Image may have **halo artifacts**.

🔹 Mnemonics 💡

- **Phase Contrast → "Phase to Face"**
    - Converts **phase differences → visible face (contrast)**.

🔹 Exam Capsule 🎯

- **Phase-contrast microscope** → converts refractive index differences into contrast.
- Components: **annular diaphragm + phase plate**.
- Best for **unstained, live cells** → shows organelles & cell processes.
- Limitation → costly, halo effect, not for thick tissues.

📄 9 – Fluorescence Microscope 🌈🔬

🔹 Principle

- Based on **fluorescence**: substance absorbs short wavelength (UV/blue) and emits longer wavelength (visible light).
- Uses **fluorochromes (dyes/probes)** → bind to specific cellular components.
- Fluorescent structures appear **brightly colored on dark background**.

🔹 Components

1. **Light Source** → UV lamp, mercury vapor lamp, LED, or laser.
2. **Exciter Filter** → selects UV/blue light.

3. **Dichroic Mirror** → reflects excitation light, transmits emitted fluorescence.
4. **Barrier Filter** → blocks unwanted light, passes emitted fluorescence.
5. **Objective Lenses** → focus & magnify specimen.

◈ Applications

- **Cell Biology** → localization of DNA, proteins, organelles.
- **Medical Diagnosis** → detection of TB (Auramine O stain), malaria, syphilis.
- **Immunology** → Fluorescent antibody technique (FITC, rhodamine).
- **Molecular Biology** → GFP (green fluorescent protein) tagging in live cells.
- **Genetics** → FISH (Fluorescence in situ hybridization).

◈ Advantages

- Highly **specific** due to selective binding of fluorochromes.
- Can detect very small quantities of molecules.
- Allows **live-cell imaging** with fluorescent proteins (GFP, RFP).

◈ Limitations

- Photobleaching → fluorochromes fade with prolonged exposure.
- Background autofluorescence may interfere.
- Requires costly filters & illumination system.

◈ Mnemonics 💡

- **Fluorescence Microscope → "LEDB"**
  - **L**ight source (UV/laser)
  - **E**xciter filter
  - **D**ichroic mirror
  - **B**arrier filter

◈ Exam Capsule 🎯

- **Fluorescence microscopy** uses **fluorochromes** + UV/blue light.
- Components → exciter filter, dichroic mirror, barrier filter.

- Applications → **medical diagnostics, immunology, molecular biology (GFP, FISH)**.
- Limitation → photobleaching, autofluorescence, high cost.

📄 10 – Electron Microscopy (Overview: TEM vs SEM) ⚡🔬

🔹 Principle

- Uses a **beam of electrons** instead of light.
- **Electrons have shorter wavelength** (0.005 nm) → much higher resolution than light microscope (~0.2 nm vs 200 nm).
- Requires **vacuum** (electrons can't travel in air).

🔹 Components

1. **Electron Source** → tungsten filament or electron gun.
2. **Electromagnetic Lenses** → replace glass lenses, bend & focus electron beam.
3. **Vacuum System** → prevents scattering of electrons.
4. **Specimen Holders & Detectors** → collect signals and form images.

🔹 Types of Electron Microscopy

1. **Transmission Electron Microscope (TEM)**

- Electrons transmitted through thin specimen.
- Produces **2D ultrastructure image**.
- Resolution: ~0.1–0.2 nm.
- Example: mitochondria cristae, ribosomes, viruses.

2. **Scanning Electron Microscope (SEM)**

- Electrons scan surface of specimen.
- Produces **3D surface image**.
- Resolution: ~5–10 nm.
- Example: insect exoskeleton, pollen grains, cell surfaces.

🔹 Applications

- Cell biology → ultrastructure of organelles.
- Microbiology → viruses, bacteria, spores.
- Materials science → surface morphology, fracture analysis.
- Medicine → pathology, nanomedicine.

🔹 **Advantages**

- Very high resolution (1000× better than light microscope).
- SEM provides 3D images.
- TEM reveals fine internal details.

🔹 **Limitations**

- Expensive & bulky.
- Requires skilled operator.
- Specimens must be dead, in vacuum, and heavily processed.
- No live-cell imaging possible.

🔹 **Mnemonics** 💡

- **TEM vs SEM → "T = Thin, Inside; S = Surface, 3D"**

🔹 **Exam Capsule** 🎯

- **Electron microscopy** → uses electron beam + electromagnetic lenses.
- **TEM** → thin section, 2D, internal ultrastructure.
- **SEM** → surface topography, 3D images.
- Resolution: TEM (0.1 nm) > SEM (5–10 nm).
- Limitation → no live cells, costly, complex prep.

📄 11 – Transmission Electron Microscope (TEM) ⚡

🔹 **Principle**

- A **beam of electrons** passes through an **ultra-thin specimen**.
- Different structures scatter electrons to different extents.

- Contrast is produced → **2D image of internal ultrastructure**.

◈ Components

1. **Electron Source** → tungsten filament (electron gun).
2. **Condenser Lens** → focuses beam onto specimen.
3. **Specimen Holder** → very thin section (~50–100 nm).
4. **Objective Lens** → magnifies electron image.
5. **Projector Lens** → further magnifies.
6. **Fluorescent screen/CCD camera** → final image capture.

◈ Sample Preparation

- Fixation (glutaraldehyde, osmium tetroxide).
- Dehydration (ethanol/acetone).
- Embedding (epoxy resin).
- Sectioning (ultramicrotome → ultrathin slices).
- Staining with heavy metals (uranyl acetate, lead citrate).

◈ Applications

- **Cell ultrastructure** → mitochondria, ER, ribosomes, chloroplasts.
- **Viruses** → morphology, infection studies.
- **Pathology** → renal biopsy, tumor analysis.
- **Nanotechnology** → nanoparticles, materials.

◈ Advantages

- Highest resolution (~0.1–0.2 nm).
- Reveals **internal fine details**.
- Can visualize **macromolecular complexes**.

◈ Limitations

- Specimen must be **ultra-thin & dead**.
- Complex sample preparation.
- High vacuum required.

- No 3D imaging (only 2D projection).
- Very expensive.

🔹 Mnemonics 💡

- **TEM → "Thin = Transmission"** (needs thin section, shows internal details).

🔹 Exam Capsule 🎯

- **TEM** uses electron beam through thin specimen.
- Produces **2D image of ultrastructure**.
- Sample prep → fixation, dehydration, embedding, ultrathin section, heavy metal staining.
- Applications → organelles, viruses, nanomaterials.
- Limitation → complex prep, only dead specimens, no 3D.

📄 12 – Scanning Electron Microscope (SEM) ⚡🔬🌐

🔹 Principle

- A **focused beam of electrons** scans across specimen surface.
- Secondary electrons emitted from surface are collected → **3D surface image**.
- Resolution: ~5–10 nm.

🔹 Components

1. **Electron Gun** → produces primary electron beam.
2. **Electromagnetic Lenses** → focus beam onto specimen.
3. **Scanning Coils** → move beam in raster pattern.
4. **Detectors** → collect emitted secondary electrons.
5. **Computer/Monitor** → reconstructs 3D image.

🔹 Sample Preparation

- Specimen fixed (glutaraldehyde).
- Dehydrated (ethanol/acetone).
- Dried (critical point drying).

- Coated with **gold/palladium** (conductive coating).

◈ Applications

- **Surface morphology** → insect cuticle, pollen grains, leaf surfaces.
- **Material science** → metals, fracture analysis, nanomaterials.
- **Forensics** → gunshot residue, fibers, soil particles.
- **Medical sciences** → bone, tissue scaffolds, implants.

◈ Advantages

- Produces **3D images**.
- Larger depth of field compared to TEM.
- Suitable for thick & bulky specimens.
- Excellent for surface detail.

◈ Limitations

- Lower resolution than TEM (~5–10 nm).
- Only surface details (no internal ultrastructure).
- Samples must be dry & conductive (coating required).
- Expensive & requires vacuum.

◈ Mnemonics 💡

- **SEM** → **"Surface Electron Microscope"** = shows external 3D structure.

◈ Exam Capsule 🎯

- **SEM** scans specimen surface with electrons → 3D image.
- Requires conductive coating (gold/palladium).
- Applications → insects, pollen, materials, forensics.
- Advantages → 3D, large depth of field.
- Limitation → lower resolution than TEM, no internal details.