BP704T · B.Pharm Semester VII · Theory Notes

Modern Analytical Techniques

Unit V — Microscopy Techniques

Unit V (5 hours)

Pills & Python Notes

From-zero teaching standard: every technical term is defined before use.

Syllabus coverage: Unit V (5 hours).

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CHAPTER 11: Microscopy-Based Analytical Techniques

Learning Objectives

By the end of this chapter, you will be able to:

1 Explain the principle of optical microscopy in simple words, including the concepts of magnification and resolution.

2 Describe the main parts of an optical microscope.

3 Explain the principle of scanning electron microscopy (SEM) simply.

4 Describe the main parts of an SEM instrument.

5 Explain the principle of transmission electron microscopy (TEM) simply.

6 Describe the main parts of a TEM instrument.

7 Compare optical microscopy, SEM, and TEM in a table (what each shows, magnification concept in general terms).

8 List pharmaceutical applications: particle size and shape, surface morphology, polymorphism support, and nanotechnology.

Definitions

- **Microscopy** — the science of viewing very small objects using a microscope, an instrument that magnifies them.
- **Magnification** — how many times larger an object appears through the microscope compared to its real size.
- **Resolution** — the ability to see two very close points as two separate points instead of one blurry blob. Better resolution = sharper, clearer detail.
- **Optical microscopy** — microscopy that uses visible light and glass lenses to magnify a sample.
- **Scanning electron microscopy (SEM)** — microscopy that scans a focused beam of electrons across the sample surface to form a highly magnified, 3D-like image of the surface.
- **Transmission electron microscopy (TEM)** — microscopy in which a beam of electrons passes *through* a very thin sample to form an image of its internal structure.
- **Electron beam** — a stream of electrons (tiny negatively charged particles) focused into a narrow beam; used instead of light in electron microscopes.
- **Morphology** — the study of the shape, form, and surface features of particles.
- **Polymorphism** — the property of a drug existing in more than one crystal form; different forms can dissolve differently.

- **Nanotechnology** — technology working with extremely small particles (nanometer scale); in pharma, used for nano-sized drug carriers.

Terminology Table

Term	Simple meaning
Microscope	Instrument that magnifies tiny objects
Magnification	How many times bigger the image looks
Resolution	Sharpness — ability to see fine detail separately
Objective lens	The lens closest to the sample; does most of the magnifying
Eyepiece	The lens you look through
Condenser	Lens system that focuses light onto the sample
Electron gun	Part that produces the electron beam in SEM/TEM
Vacuum	Empty space with air removed; needed inside electron microscopes
Sputter coating	Coating the sample with a very thin layer of metal (for SEM) so it conducts electrons
Ultrathin section	An extremely thin slice of sample needed for TEM
Surface morphology	The shape and texture of a particle's surface

Simple Explanations

Concept 1: Optical microscopy — principle

An **optical (light) microscope** works like a powerful magnifying glass made of several lenses:

- Visible **light** shines through (or onto) the sample.
- Glass **lenses** bend the light to form a much larger image of the tiny object.
- **Magnification** tells you how many times larger the image is. Total magnification = objective lens magnification × eyepiece magnification.
- **Resolution** is about sharpness, not just size. You can enlarge a blurry photo as much as you like — it stays blurry. A microscope is useful only if it can *resolve* (separate) fine details. With visible light, there is a natural limit to how fine the detail can be, because light itself has a certain wavelength.

Simple takeaway: Optical microscopy = light + lenses → bigger image. Magnification = size; resolution = sharpness.

Concept 2: Parts of an optical microscope (instrumentation)

1. **Light source** — a lamp at the base that provides light.
2. **Condenser** — lenses that collect the light and focus it onto the sample as a bright, even beam.
3. **Stage** — the flat platform where the sample slide is placed.

4 **Objective lens** — the lens just above the sample; does most of the magnifying. Several objectives of different strengths are mounted on a rotating nosepiece.

5 **Eyepiece (ocular)** — the lens you look through; magnifies the image further.

6 **Focus knobs** — coarse and fine adjustment knobs that move the stage or lenses to get a sharp image.

Light path: Lamp → condenser → sample on stage → objective lens → eyepiece → your eye.

Concept 3: SEM — principle (simply)

A **scanning electron microscope** does not use light at all — it uses **electrons**:

- An **electron gun** produces a narrow beam of electrons.
- This beam **scans** (moves back and forth, like reading lines on a page) across the **surface** of the sample.
- Where the beam hits, the sample gives off signals (secondary electrons); a detector collects them point by point.
- A computer builds these points into a highly magnified image that looks **three-dimensional** — you can see the surface texture, bumps, and shape of particles.
- Because electrons are much smaller than light waves, SEM can show far finer detail than an optical microscope.
- The inside of the SEM is kept under **vacuum** (air removed) so electrons travel without hitting air molecules.
- Samples usually get a very thin **metal coating** (sputter coating) so the electron beam does not build up electric charge on the surface.

Simple takeaway: SEM = electron beam scans the surface → 3D-like picture of surface shape and texture, at much higher magnification than light microscopes.

Concept 4: Parts of an SEM (instrumentation)

1 **Electron gun** — produces the electron beam.

2 **Electromagnetic lenses** — magnetic coils that focus the electron beam (like glass lenses focus light, but using magnets).

3 **Scan coils** — move the beam back and forth across the sample surface.

4 **Sample chamber (with stage)** — holds the sample (usually metal-coated) under vacuum.

5 **Detector** — collects the electrons/signals coming off the sample surface.

6 **Display/computer** — converts the signals into the magnified image on screen.

7 **Vacuum system** — pumps that remove air from inside the instrument.

Concept 5: TEM — principle (simply)

A **transmission electron microscope** also uses electrons, but differently from SEM:

- The electron beam **passes through** (is transmitted through) a **very thin** sample — like light passing through a thin slide, but with electrons.

- Denser/thicker parts of the sample block more electrons; thinner/lighter parts let more through.
- The transmitted electrons form an image showing the **internal structure** of the sample.
- TEM gives the finest detail of all three microscopes — it can reveal internal features at near-atomic scale.
- The sample must be extremely thin (an ultrathin section), otherwise electrons cannot pass through.
- Like SEM, the instrument works under vacuum.

Simple takeaway: TEM = electrons pass through a super-thin sample → picture of the *inside* structure with the finest detail.

Concept 6: Parts of a TEM (instrumentation)

1 **Electron gun** — produces the electron beam.

2 **Condenser lens system (electromagnetic)** — focuses the beam onto the sample.

3 **Sample holder** — holds the ultrathin sample section.

4 **Objective lens (electromagnetic)** — forms the first magnified image from transmitted electrons.

5 **Projector lenses** — magnify the image further.

6 **Fluorescent screen / camera** — where the final image is viewed or recorded.

7 **Vacuum system** — keeps air out of the column.

Concept 7: Optical vs SEM vs TEM (comparison concept)

- **Optical microscopy** shows the general appearance of samples with light; moderate magnification; simplest and cheapest; samples need little preparation.
- **SEM** shows the **surface** — shape, texture, 3D-like views; much higher magnification than optical; needs vacuum and usually metal coating.
- **TEM** shows the **inside** — internal structure through ultrathin sections; highest magnification and finest detail of the three; most demanding sample preparation.

(Exact magnification numbers vary with instrument model, so they are not fixed here — what matters is the order: optical < SEM < TEM in magnifying power and detail.)

Classifications

Classification of microscopy techniques in this chapter

```
MICROSCOPY-BASED ANALYTICAL TECHNIQUES
├── Optical (light) microscopy
│   └── Uses visible light + glass lenses; shows general appearance
├── SEM (Scanning Electron Microscopy)
│   └── Electron beam scans the SURFACE; 3D-like surface images
└── TEM (Transmission Electron Microscopy)
    └── Electron beam passes THROUGH thin sample; internal structure images
```

Mechanisms / Processes

How an SEM forms an image (step by step)

1 The electron gun fires a focused electron beam in a vacuum chamber.

2 Scan coils sweep the beam across the sample surface, point by point, line by line.

3 At each point, the beam knocks signals (electrons) out of the sample surface.

4 The detector collects these signals; stronger signal = brighter point.

5 The computer assembles all points into a magnified, 3D-like surface image on screen.

How a TEM forms an image (step by step)

1 The electron gun fires a beam through electromagnetic condenser lenses.

2 The beam passes through the ultrathin sample; dense regions block electrons, thin regions let them through.

3 Transmitted electrons pass through objective and projector lenses, which magnify the image.

4 The final image appears on a fluorescent screen or camera, showing internal structure.

Textual Diagrams / Block Diagrams

Diagram 1: Optical microscope — labeled block diagram

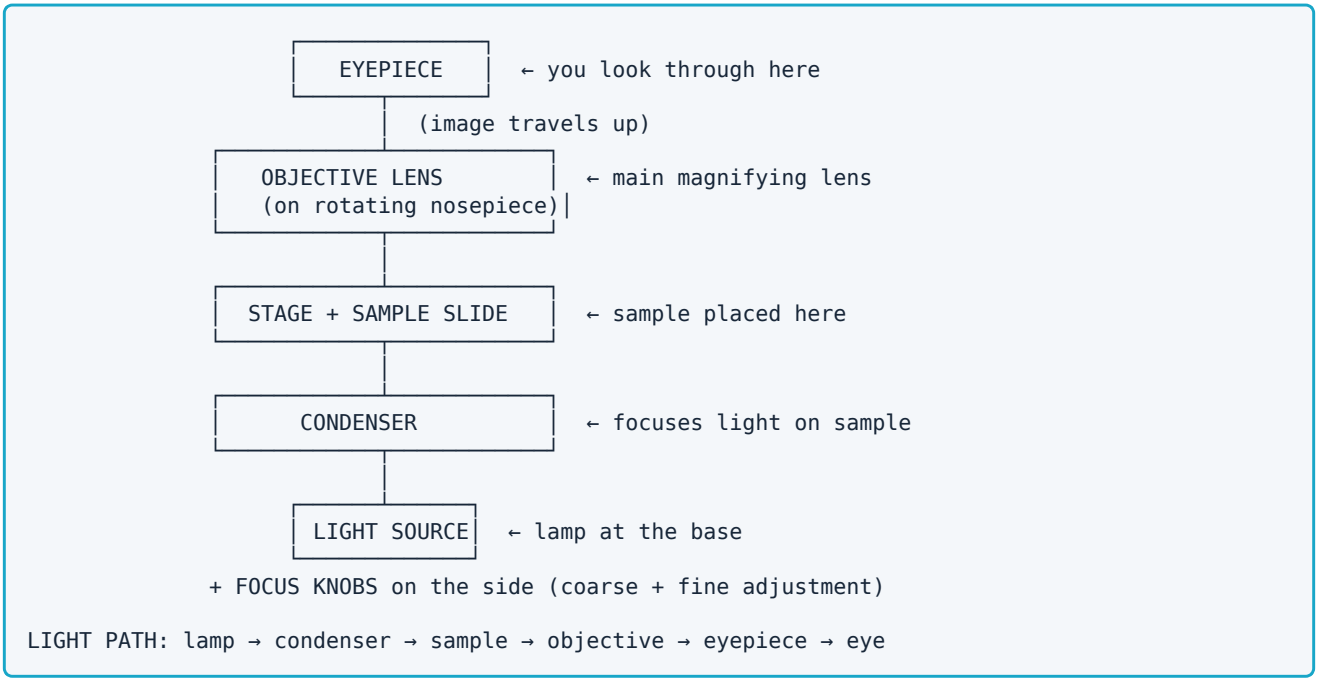


Diagram 2: SEM — labeled block diagram

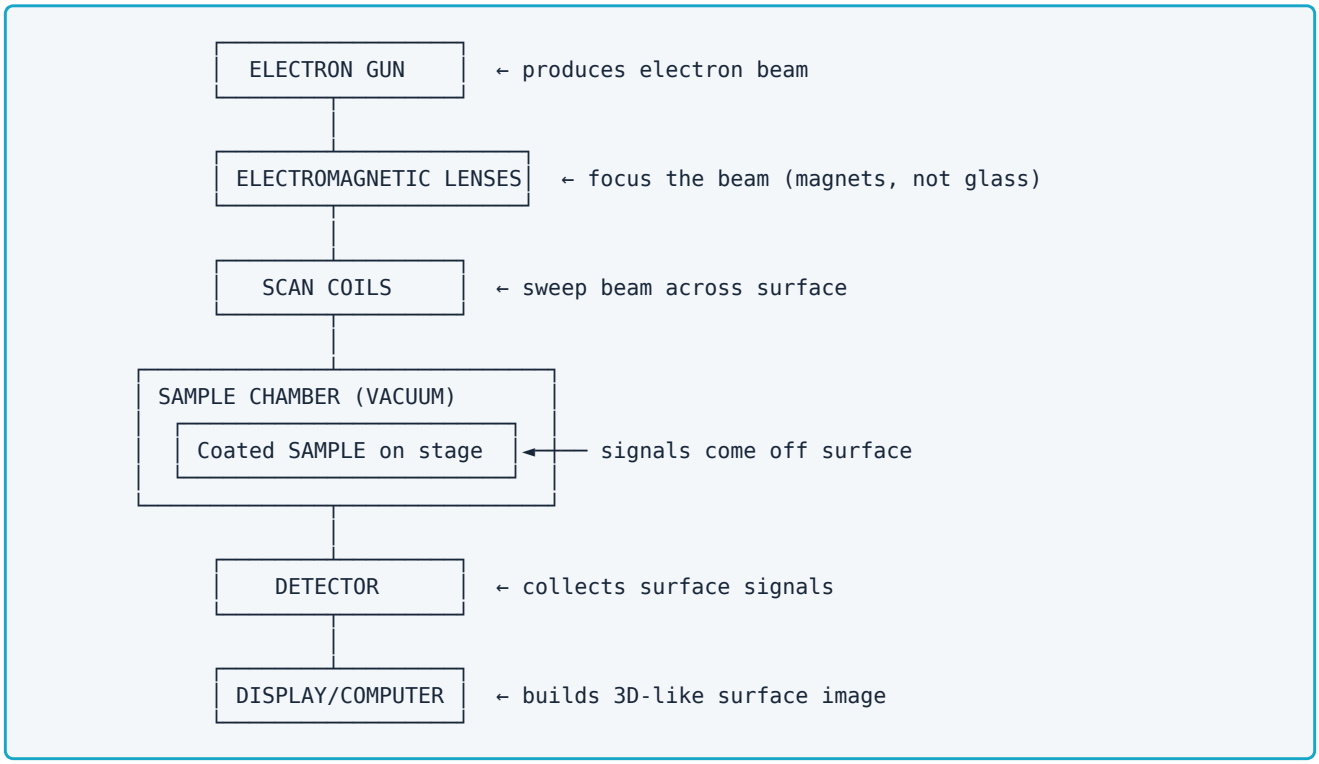
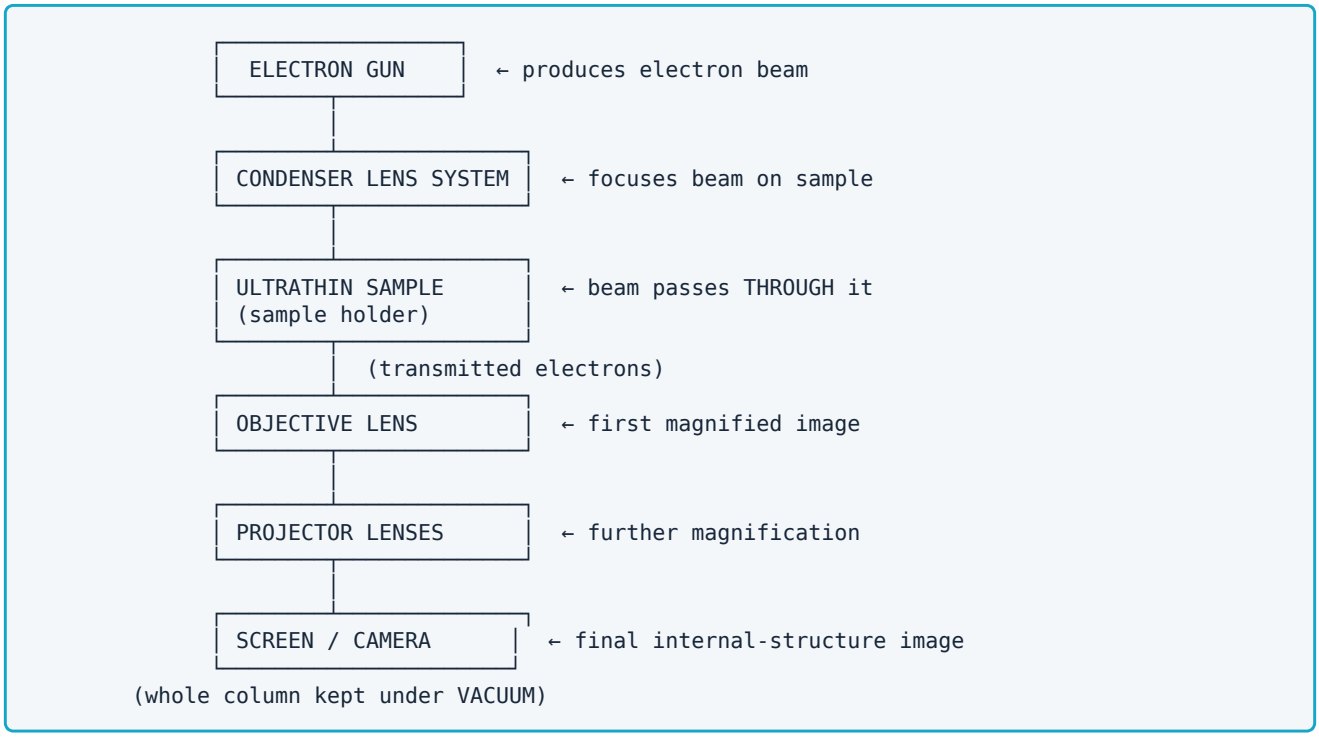


Diagram 3: TEM — labeled block diagram



Flowcharts

Flowchart 1: Choosing the right microscope

```
START: what do you need to see?
        |
        v
Is a general magnified view of the sample enough?
   ├── YES → use OPTICAL microscopy (simplest, cheapest)
   └── NO → continue
        |
        v
Do you need to see the SURFACE shape and texture in 3D-like detail?
   ├── YES → use SEM (coat sample with thin metal, work under vacuum)
   └── NO → continue
        |
        v
Do you need to see the internal structure in finest detail?
   └── YES → use TEM (prepare ultrathin section, work under vacuum)
```

Flowchart 2: SEM sample workflow

```
START: powder/particle sample
        |
        v
Mount sample on holder → apply thin metal coating (sputter coating)
        |
        v
Place in vacuum chamber → pump out air
        |
        v
Scan electron beam across surface → detector collects signals
        |
        v
Computer builds magnified 3D-like surface image
```

Infographic Concept Box

INFOGRAPHIC CONCEPT: A single-page visual titled "THREE WINDOWS INTO THE TINY WORLD". Three large panels side by side: (1) an eye looking through a classic microscope labeled "OPTICAL — light + lenses, general view"; (2) a beam sweeping over a bumpy particle surface labeled "SEM — scans the SURFACE, 3D-like texture"; (3) a beam passing through a thin slice labeled "TEM — sees the INSIDE, finest detail". Above the panels, three magnifying glasses of increasing size showing the order of magnifying power: optical < SEM < TEM. Bottom strip: four application icons — a powder particle (particle size/shape), a textured sphere (surface morphology), two different crystal shapes (polymorphism), and tiny spheres in a capsule (nanotechnology). Footer: "Light shows the outside simply; electrons reveal the hidden details."

Tables

Table 1: Optical vs SEM vs TEM comparison

Feature	Optical microscopy	SEM	TEM
What is used to "see"	Visible light	Electron beam	Electron beam
What is shown	General appearance of sample	Surface shape and texture (3D-like)	Internal structure

Feature	Optical microscopy	SEM	TEM
Magnifying power (general order)	Lowest of the three	Much higher than optical	Highest of the three
Resolution/detail	Moderate (limited by light)	Fine surface detail	Finest internal detail
Sample preparation	Simple (slide)	Metal coating + vacuum	Ultrathin section + vacuum
Cost and complexity	Simplest, cheapest	More complex	Most complex

Table 2: Parts and their functions

Instrument	Part	Function
Optical	Condenser	Focuses light onto the sample
Optical	Objective lens	Main magnification
Optical	Eyepiece	Final magnification for viewing
SEM	Electron gun	Produces the electron beam
SEM	Scan coils	Sweep the beam across the surface
SEM	Detector	Collects signals from the surface
TEM	Condenser system	Focuses beam onto the thin sample
TEM	Objective + projector lenses	Magnify the transmitted image
TEM	Screen/camera	Displays the final image

Examples

1. **Optical microscopy:** Checking a powder sample to see the general shape of particles and whether foreign particles are mixed in.
2. **SEM:** Looking at a tablet's coating surface to see if the coating is smooth or cracked — the 3D-like image clearly shows cracks and roughness.
3. **TEM:** Examining nano-sized drug carrier particles to confirm their internal structure and that the drug is properly loaded inside.
4. **Polymorphism support:** Two crystal forms of the same drug look different under the microscope (needles vs plates) — microscopy supports other techniques in confirming which form is present.
5. **Particle size/shape:** Comparing granules before and after milling — microscopy shows whether milling actually made the particles smaller and more uniform.

Analogies

- **Magnification vs resolution is like zooming a blurry photo.** Zooming (magnification) makes it bigger but not clearer; only a sharper original photo (better resolution) shows more detail.
- **SEM scanning is like reading a book line by line.** The beam moves across the surface row by row, collecting information point by point, and the computer assembles the full picture — just as your eyes assemble lines into a page.

- **TEM is like an X-ray of luggage at the airport.** The beam passes through and shows what is *inside*, not just the surface of the bag.
- **Sputter coating for SEM is like painting a plastic chair with metallic paint** so it can carry current — the thin metal layer lets the electron beam work without charge building up.
- **The three microscopes are like three pairs of glasses:** reading glasses (optical) for everyday viewing, binoculars (SEM) for distant surface detail, and a super-telescope (TEM) for the finest hidden structure.

Pharmacy Relevance

- Particle size and shape directly affect how a tablet dissolves and how a powder flows in manufacturing — microscopy lets pharmacists actually *see* these properties.
- Tablet coating defects (cracks, uneven coating) found by SEM explain why some tablets release drug too fast or too slow.
- Different crystal forms (polymorphs) of the same drug can have different solubility — microscopy helps identify which form is in the batch, protecting drug quality.
- Nanotechnology-based medicines (nano-sized carriers that deliver drugs to specific body parts) are developed and checked using SEM and TEM.
- Pharmacists in quality control and research use these images as visual proof in reports and investigations.

India Context

- Indian pharmaceutical companies manufacturing tablets, capsules, and nano-formulations use SEM/TEM in their R&D centers to study particle shape, coating quality, and nano drug carriers.
- Government and university research institutes across India provide electron microscopy facilities where pharmacy researchers can analyze their samples.
- As Indian companies develop more complex products (coated tablets, nano-medicines, herbal extracts), microscopy skills make pharmacy graduates more employable in formulation R&D.
- Optical microscopes remain the everyday workhorse in Indian QC labs and colleges — simple, affordable, and sufficient for routine particle and crystal checks.

Key Points

- Optical microscopy: light + glass lenses; shows general appearance; magnification = size, resolution = sharpness.
- SEM: electron beam scans the surface; 3D-like surface images; much higher magnification than optical; needs vacuum and metal coating.
- TEM: electron beam passes through an ultrathin sample; shows internal structure; finest detail of the three.
- Order of magnifying power: optical < SEM < TEM.

- Applications: particle size/shape, surface morphology, polymorphism support, nanotechnology in pharma.

Common Confusions

- **SEM vs TEM:** SEM looks at the *surface* (beam scans across it); TEM looks at the *inside* (beam passes through a thin section). Remember: **S**EM = **S**urface, **T**EM = **T**ransmission (through).
- **Magnification vs resolution:** Bigger image (magnification) is useless without sharpness (resolution). Students often think higher magnification automatically means seeing more — it does not, unless resolution also improves.
- **"Electron microscopes use light."** No — they use electron beams focused by electromagnetic (magnetic) lenses, not glass lenses and light.
- **Why vacuum?** Air molecules would collide with the electrons and scatter the beam — so air must be pumped out.

Common Mistakes

- Putting a thick sample in TEM — electrons cannot pass through thick samples; ultrathin sections are a must.
- Forgetting metal coating in SEM — uncoated non-conducting samples build up charge and give distorted, bright-blotched images.
- Touching the objective lens or eyepiece carelessly — optical lenses scratch easily and are costly to replace.
- Interpreting SEM surface features as internal structure — SEM only shows the surface; internal claims need TEM.
- Quoting exact magnification numbers from memory in exams — magnification ranges differ by instrument; describe the general order (optical < SEM < TEM) instead of memorized figures.

Mnemonics

- **"S for Surface (SEM), T for Through (TEM)"** — the one-line way to never confuse them.
- **"Light Lenses Look"** — optical microscopy: **L**ight + **L**enses → **L**ook at general appearance.
- **Magnification vs resolution:** "**Big** is magnification, **sharp** is resolution."
- **Order of power:** "**O-S-T** — **O**ptical < **S**EM < **T**EM" (alphabetical order matches increasing power).

Exam Summary

Microscopy-based techniques let analysts see what chemical tests cannot — the size, shape, surface, and internal structure of particles. Optical microscopy uses visible light and glass lenses (condenser, objective, eyepiece) to magnify samples; magnification describes image size while resolution describes sharpness. Scanning electron microscopy scans a focused electron beam across the sample surface under vacuum,

building a highly magnified 3D-like image of surface morphology; samples usually receive a thin metal coating. Transmission electron microscopy passes the electron beam through an ultrathin sample to image internal structure with the finest detail of the three techniques. In general, magnifying power increases in the order optical < SEM < TEM. Pharmaceutical applications include studying particle size and shape, surface morphology (e.g., tablet coating quality), supporting polymorphism studies, and developing nanotechnology-based drug delivery systems.

One-Minute Revision

- Optical: light + lenses; general view; big = magnification, sharp = resolution.
- SEM: electron beam scans SURFACE → 3D-like texture; vacuum + metal coating.
- TEM: electrons pass THROUGH ultrathin sample → internal structure; finest detail.
- Power order: Optical < SEM < TEM.
- Uses: particle size/shape, surface morphology, polymorphism, nanotechnology.
- S = Surface (SEM); T = Through (TEM).

Short Answer Questions

1 Define magnification and resolution.

2 What is the light path in an optical microscope?

3 Name the main parts of an optical microscope with one-line functions.

4 What does SEM stand for, and what does it show?

5 Why is vacuum needed in SEM and TEM?

6 What is sputter coating, and why is it done in SEM?

7 What does TEM stand for, and what does it show?

8 Why must the TEM sample be ultrathin?

9 State the general order of magnifying power among optical, SEM, and TEM.

10 Give two pharmaceutical applications of SEM.

11 Give one pharmaceutical application of TEM.

12 How does microscopy support polymorphism studies?

Long Answer Questions

1 Explain the principle and instrumentation of optical microscopy with a labeled block diagram. Clarify magnification vs resolution.

2 Explain the principle and instrumentation of SEM with a labeled block diagram. Describe how the image is formed.

3 Explain the principle and instrumentation of TEM with a labeled block diagram. Why is sample preparation demanding in TEM?

4 Compare optical microscopy, SEM, and TEM with respect to radiation used, what is observed, sample preparation, and magnifying power.

5 Discuss the applications of microscopy-based techniques in pharmacy: particle size/shape, surface morphology, polymorphism, and nanotechnology.

MCQs with Answer Keys

1 Optical microscopy uses:

a) Electron beam b) Visible light and glass lenses c) X-rays d) Sound waves **Answer: b**

1 Resolution means:

a) How big the image looks b) The ability to see two close points as separate (sharpness) c) The color of the image d) The weight of the microscope **Answer: b**

1 In an optical microscope, the lens that does most of the magnifying is the:

a) Eyepiece b) Objective lens c) Condenser d) Mirror **Answer: b**

1 SEM forms an image by:

a) Passing electrons through a thin sample b) Scanning an electron beam across the sample surface c) Shining visible light through lenses d) Heating the sample **Answer: b**

1 SEM images typically appear:

a) Flat and 2D only b) 3D-like, showing surface texture c) Invisible d) Black and white text **Answer: b**

1 Vacuum is needed in electron microscopes because:

a) Electrons need darkness b) Air molecules would scatter the electron beam c) Samples melt in air d) Lenses fog up **Answer: b**

1 Sputter coating in SEM is done to:

a) Color the sample b) Apply a thin metal layer so charge does not build up c) Dissolve the sample d) Magnify the image **Answer: b**

1 TEM shows mainly the:

a) Surface texture b) Internal structure (beam passes through a thin sample) c) Color of the sample d) Smell of the sample **Answer: b**

1 The correct order of increasing magnifying power is:

a) TEM < SEM < optical b) Optical < SEM < TEM c) SEM < optical < TEM d) All are equal **Answer: b**

1 "S for Surface, T for Through" helps remember:

a) SPE vs PPE b) SEM vs TEM c) RIA vs ELISA d) PK vs PD **Answer: b**

1 Which technique best shows cracks in a tablet coating?

a) TEM b) SEM c) Naked eye only d) pH meter **Answer: b**

1 Microscopy supports polymorphism studies by:

a) Measuring melting point b) Showing different crystal shapes of the same drug c) Counting tablets d) Testing dissolution directly **Answer: b**

Viva Questions

1 What is the difference between magnification and resolution?

2 Trace the light path in an optical microscope.

3 What does SEM scan — surface or interior?

4 Why must TEM samples be ultrathin?

5 Why is air removed from electron microscopes?

6 What is the function of scan coils in SEM?

7 Which gives finer detail — SEM or TEM?

8 Give one use of microscopy in tablet manufacturing.

9 How is microscopy useful in nanotechnology?

10 Why can't we use glass lenses in electron microscopes?

Application-Based Questions

1 A batch of coated tablets is releasing drug too fast. Which microscopy technique would you use to investigate, what would you look for, and why did you choose that technique?

2 Your R&D team is developing nano-sized drug carriers. Which technique (SEM or TEM) would confirm the internal drug loading, and which would check surface shape? Justify.

3 Two batches of the same drug show different dissolution rates, and polymorphism is suspected. How would microscopy help, and what would you expect to see?

4 A student puts a thick powder mound directly into a TEM and gets no image. Explain what went wrong and how to prepare the sample correctly.

5 An SEM image of an uncoated tablet sample shows bright distorted patches. Diagnose the likely cause and the corrective step.

References

- Brown
- Cullity & Stock
- Dong

- Friebolin
- Niessen
- Poole
- Silverstein/Webster/Kiemle/Bryce
- Skoog/Holler/Crouch