Microscope and Microscopy

9.1. What is a Microscope?

You have read in Grade 8 that a special instrument called the **microscope** (*micro* – small; + *skopion* - "means of viewing") is required to observe tiny living organisms or their parts which cannot be seen through naked eyes by magnifying them. With a microscope, you can see small specimens such as onion cells, cheek cells, bacteria and even dust particles etc. It helps doctors to see germs and study cells in living organisms.

What do we call the ability of a human eye to see two very close objects as separate and distinct? Imagine two tiny dots drawn on a piece of paper. As the dots are moved closer, there comes a point at which they can no longer appear as separate. When viewed from about 25 cm (the near point of the eye), two points separated by about 0.1 mm (100 µm) can be observed as distinct, otherwise, they appear as a single point. This defines the **limit of resolution** of the human eye.

A cell is generally too tiny to be observed by an unaided eye. This raises an important question - how do cell biologists study the structure and functioning of cells, which are much smaller than the limit of resolution of the human eye?

When Robert Hooke observed 'cork' under the microscope developed by him in 1665. He examined thin slices of bark of an oak tree and observed tiny hexagonal box-like spaces just like the patterns of honeycomb and called them **cells**. Around the same time, Antony van Leeuwenhoek made tiny, powerful lenses and saw "animalcules" – what we now know as bacteria and protozoa. Those simple lenses opened the door to a completely new world.

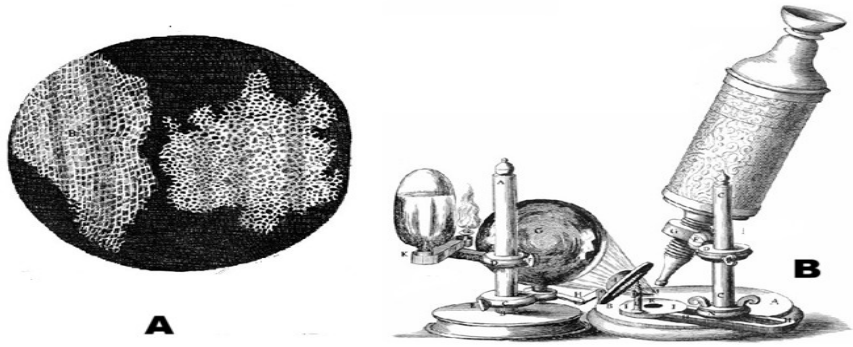


Figure 9.1: A. Drawings of Cork cells as published in the '*Micrographia*'; B. Microscope developed by Robert Hooke,

Activity 9.1: Let us think and write:
If you could shrink yourself and travel inside a leaf, what would you see? Write 3 – 4 lines imagining that journey.

9.2. A Quick historical Journey of Microscopes

Let us walk through time and see how microscopes evolved:

- **13th–15th century:** Simple magnifying glasses used by spectacle makers.
- **1590 - Hans and Zacharias Janssen:** A Dutch father-and-son duo of spectacle makers developed an early compound microscope by combining two lenses within a single tube.
- **1665 – Robert Hooke:** Coined the term 'cell' for empty, hexagonal, box-like structures by examining the cork of an oak tree under the microscope developed by him. He published his findings in a book called "Micrographia".
- **1670s – Antony van Leeuwenhoek:** He worked with a simple, single-lens microscope capable of magnifying up to about 300 times, which allowed him to observe tiny living organisms he called "animalcules," including bacteria and protozoa. He was the first to study living microorganisms and is widely regarded as the ***Father of Microscopy.***
- **1878 Ernst Abbe:** Postulated a mathematical theory linking resolution to the wavelength.
- **19th–20th century:** Better lenses and illumination improved the compound light microscope.
- **1930s onwards: Electron microscopes** (TEM and SEM) were invented where viruses, cell organelles and cell surfaces could be observed.
- **1938 Ernst Ruska:** developed the first electron microscope, which operated on the principle using electrons as the illumination source (instead of light) that provides shorter wavelengths and thereby significantly enhancing the resolving power.
- **1953**: **Frits Zernike** received the Nobel Prize in Physics for inventing and demonstrating the phase-contrast microscope.

Activity 9.2: A Timeline Strip

Draw a horizontal line. Mark at least 5 important dates in microscopy and add a tiny sketch or symbol for each (e.g., cork cells, bacteria, electron beam etc.).

9.3. How Does a Microscope Work?

An important parameter in microscopy is the **resolution, contrast** and **magnification** of the object that is viewed under the lens, which makes it appear several times larger to the human eye. The operating principle varies with the type of microscope, which can be broadly classified by whether they use multiple lenses or electron beams. In each case, a system of lenses or electromagnetic fields is used to produce an enlarged, detailed image of a specimen that cannot be clearly seen with the naked eye.

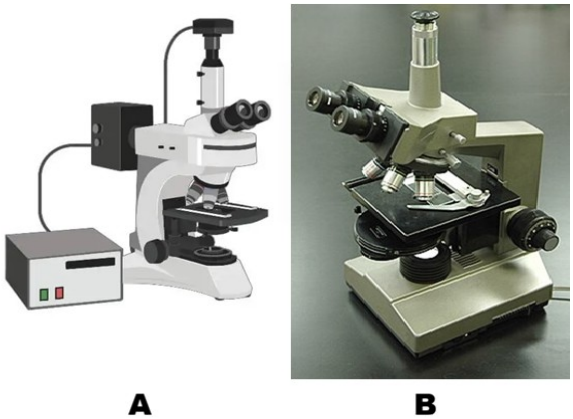
9.3.1 Types of Light microscope

Light (Optical) microscopes rely on visible light and glass lenses to enlarge and view specimens.

Basic Classification -

- **A simple microscope** utilizes a single lens to magnify an object, similar to how a magnifying glass works. For example, dissecting microscope is used for 3D viewing of small objects.
- **Compound Microscope**: Most commonly used laboratory microscope utilizes at least two sets of lenses - the objective lens (near the specimen) and the eyepiece (ocular lens) - to achieve high magnification.
- **Advanced optical microscopes**

Beyond the standard compound microscope, a diverse family of advanced light microscopes exists—such as Phase-Contrast and Fluorescence, each using unique optical technique to reveal hidden cellular secrets that would otherwise remain invisible to the naked eyes. **Fluorescence microscopy** uses high-intensity light to excite specialized dyes in a specimen, causing specific cellular structures to glow brilliantly against a dark background like stars in the night sky. **Phase-Contrast** microscopy is used for viewing **living cells** in their natural state because it enhances contrast without the need for chemical stains that would otherwise kill the specimen.



A B

Figure 9.2: A. Fluorescence microscope; B. Phase contrast microscope

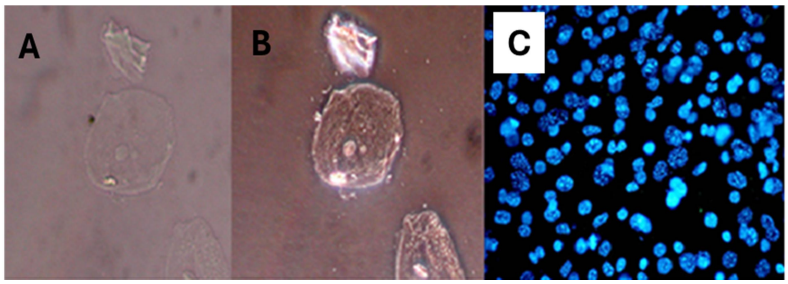


Figure 9.3: Cells imaged with – A. Traditional optical microscope (Mag. 40X) B. Phase-contrast microscope (Mag. 40X) and C. Fluorescence microscope (Mag. 20X)

9.3.2 Parts of a Compound microscope

Core Components and their Roles

1. **Light Source**; Provides illumination (LED or halogen lamp).
2. **Condenser Lens**: Focuses light onto the specimen to optimize numerical aperture and contrast.
3. **Specimen Stage**: Holds the slide containing the specimen.
4. **Objective Lens**: The primary magnifying lens (e.g., 4X, 10X, 40X, 100X). It forms an enlarged, inverted image of the specimen that is real in nature.
5. **Eyepiece (Ocular Lens)**: It enhances the magnification of the image already produced by the objective lens.

Activity 9.3: Let us examine a compound microscope

Key parts include eyepiece, objectives, nosepiece, stage with clips, coarse/ fine focus, condenser, iris diaphragm, illuminator, arm, and base

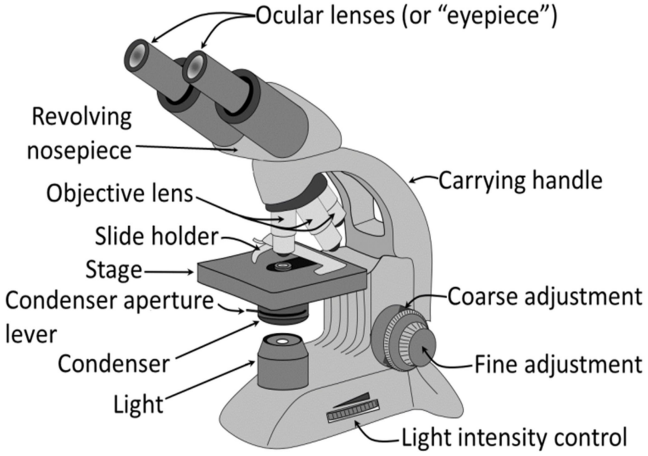


Figure 9.4: Light (compound) microscope

- **Objective lenses:** Main magnifying lenses (with different magnification power (X) – 4, 10, 15, 20, 40 etc.) close to the slide. These form a real, magnified image of the object.

- **Eyepiece (ocular lens):** Where you place your eye. It acts like a magnifying glass for the image formed by the objective. Together, these lenses produce a greatly enlarged image for your eye.
- **Body tube:** A hollow tube which has the eye piece and objective fitted on its two ends.
- **Revolving nosepiece:** Holds objectives and allows you to switch between them.
- **Stage and clips/ mechanical stage:** Platform to hold the slide in place.
- **Condenser Lens:** Consists of convex lens to focus and concentrate the light on specimen to optimize numerical aperture and contrast.
- **Substage diaphragm:** Controls the amount of light transmitted on specimen
- **Coarse adjustment knob:** Big knob for rough focusing (low power).
- **Fine adjustment knob:** Small knob for sharp focus (high power).
- **Arm and base:** Support; always hold microscope by the arm and support the base.
- **Light source/ mirror:** Provides light that passes through specimen/reflects light.

9.3.3 Light Microscope – working

Light changes direction when it passes through glass. You have earlier learnt in Grade 8 that a **convex lens** (converging lens) bends light rays to meet at a point. When we place a tiny object near such a lens, the lens forms a larger, inverted image. Light microscopes function by using refraction—the bending of light as it moves between different media due to changes in speed—along with reflection to direct and focus light rays. The objective lens, which has a short focal length, first creates a real and inverted image of the specimen placed just outside its focal point. This image then becomes the input for the eyepiece, which has a longer focal length and further enlarges it, producing the final upright, highly magnified virtual image.

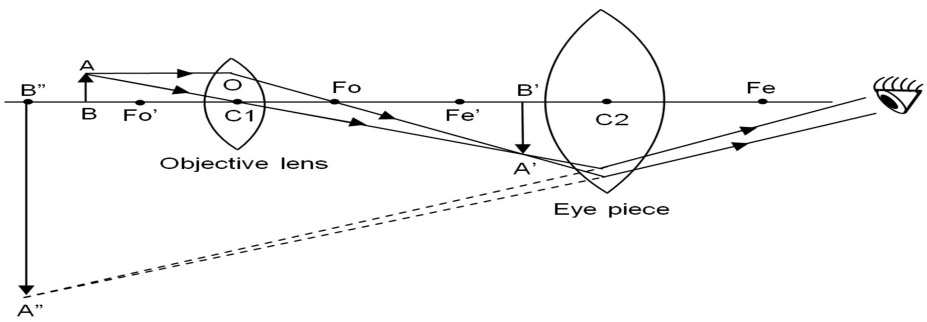


Figure 9.5: Ray diagram of compound microscope

9.4. Microscopy Skills

9.4.1 Slide Preparation and Focusing (temporary mount)

You have already learnt the method for preparing a temporary mount of an onion peel; using a similar approach, let us now create a few more temporary mounts.

Activity 9.4: Let us prepare, observe and compare leaf peels of monocot and dicot leaves:

Take peels from both upper and lower epidermis of a monocot leaf (Rhoeo/ maize/ lily) and a dicot leaf (*Bryophyllum*/ petunia/ balsam). Prepare their temporary mounts. Observe them under microscope, compare their structure and draw labelled diagrams. Record similarities and differences, if any.

Now corelate the points noted by you in activity 9.1 with your observations.

S. No.	Feature	Monocot leaf		Dicot leaf	
		Name of source plant……		Name of source plant…...	
1.	Shape of epidermal cells				
2.	Pattern of epidermal cells				
3.	Shape of Guard cells				
4.	Distribution of stomata				
5.	Any other observation				

9.4.2 Permanent Slides

We have already learnt how to prepare temporary mounts. These are useful only for a short time because the drop of water slowly dries up and the living cells start shrinking, and dying.If you leave a temporary mount of an onion peel for a few hours, you will notice air bubbles, crystals of the dried stain, and distorted cell shapes instead of fresh, clear cells.

Now think, what if we want to keep a well prepared slide of onion cells or cheek cells safe so that many future batches of students in your school can observe it? For this purpose, biologists make **permanent mounts**. The slides stored carefully in slide boxes in your laboratory are usually permanent slides that have been prepared using a special technique and preserved for years.

In a **permanent mount**, the specimen is first fixed (to kill and preserve its structure), then stained, dehydrated, and finally sealed in a special mounting medium such as Canada balsam or DPX under a coverslip. This prevents the

specimen from drying, rotting, or being attacked by microorganisms. Once prepared properly, a permanent slide can be stored in the laboratory and used repeatedly without losing clarity of cells when seen under the microscope.

Activity 9.5: Let us observe permanent slides of leaf peel of a monocot and dicot leaf

Observe a permanent slide of leaf peel of monocot and dicot leaf and compare it with the temporary mount prepared by you.

Do you find any difference in the clarity of the slides? Notice the cell walls and guard cells in the fresh temporary mount and the stained permanent mount of the dicot peel? Does the clarity of cell walls and guard cell differ? Identify two common anatomical differences in leaf peels that remain consistent in temporary and permanent mount.

9.4.3 By what factor is the image larger than the actual object?

When you look at a diagram of a cell or a tiny insect in your book, it often appears huge on the page, now you know that it might be smaller than a grain of sand. Further, when you observed onion peel cells under the microscope you would have noticed the difference in the size of image when we switch from low power to high power objective lens.

How do you know *exactly* how many times bigger you are seeing the image? We can find out by finding the magnification of objective and eye piece. Magnification is represented by the symbol X, which is read as “times,” for example, 10X means “magnified ten times.”

Magnification of a microscope: $M = m_o \times m_e$
Where m_o= magnification of objective, m_e= magnification of eyepiece.

Example:

- 10X eyepiece and 40x objective $\rightarrow M = 10 \times 40 = 400$.
- Real size = image size / total magnification

Let us find out:

1. If a cell measures 5 mm on 100X image, calculate its actual size.
2. If you use a 15X eyepiece and 10X objective, what will be the total magnification?
3. If 4 cells fit across a 0.8 mm field of view, what will be the approximate size of one cell?

9.5. Types of Microscopes

All microscopes do the same basic function – they magnify small objects – but they do it in different ways and to different depths. Magnification and resolution are core concepts in microscopy that define the imaging capabilities of a microscope. Different microscopes have different magnification powers and resolution ranges; the size of the specimen and purpose of observation determines which microscope can be used to view the specimen effectively.

9.5.1 Magnification *vs.* Resolution – Big *vs.* Sharp image

Generally, students think: "More magnification is always better." Not always true!

- **Magnification**: Magnification is the factor by which a microscope enlarges the image of a specimen relative to its actual size (e.g., 400x).
- **Resolution**: Resolution is the smallest distance between two very close points in a specimen to clearly distinguish them as separate and not merged into a single image. It is often expressed as a unit of length e.g. 0.2 µm for light microscopes. Higher resolution reveals fine details and gives a sharp image. **Resolving power** is the capacity of the microscope to clearly identify two very closely placed points as separate points. It is expressed as the reciprocal of resolution (smaller resolution means higher resolving power).

The **resolution** of human eye and some microscopes is given below:

Instrument	Resolution (in metre
Human eye	~ **1 × 10^{-4} m** (≈ 0.1 mm)
Light microscope	~ **2 × 10^{-7} m** (≈ 0.2 µm)
Electron microscope	~ **2 × 10^{-10} m** (≈ 0.2 nm)

Let's know more about the units of length used in microscopy:
1 cm = 10 mm
1 mm = 1,000 µm (micrometres)
1 µm = 1,000 nm (nanometres)

9.5.2 Light (Compound) Microscope

This is the microscope you generally use in school laboratories.

- It uses **visible light** and **glass lenses**.
- Its magnification is usually up to about 1000x.
- Its resolution (smallest detail you can see clearly) is about 0.2 µm.
- It can be used to observe living cells, like moving protozoa or cheek cells.

9.5.3. Electron Microscopes

- Electron microscopes operate with electron beams that have extremely short wavelengths (about 0.005 nm compared to 550 nm for visible light). These electrons are accelerated in a vacuum and directed using magnetic lenses.
- Electrons also behave like waves, but with a **much shorter wavelength** than visible light. A shorter wavelength gives better resolution.
- Electromagnets act as "lenses" to focus on the electron beam.
- Source of electron beam (Tungsten filament).

9.5.3.1 Transmission Electron Microscope (TEM)

It is much more powerful as compared to the light microscope because it has better magnification and resolution.

- It operates with a beam of **electrons** rather than light.
- Extremely thin slices of the specimen, about 50–90 nm thick, are prepared using an ultramicrotome equipped with a glass knife.
- Electrons pass through an ultra-thin slice of specimen.
- Heavy metal stains like uranyl acetate (for proteins/ nucleic acids) and lead citrate (for lipids/ carbohydrates) are used. These increase electron density *via* positive staining, providing contrast without dissolving in embedding resins.
- Internal details of cells – mitochondria, ribosomes, viruses etc. can be observed.
- Image produced is two – dimensional (2D).
- Resolution can be around 0.1 nm.

9.5.3.2 Scanning Electron Microscope (SEM)

- It operates with a beam of **electrons** rather than light.
- Unlike TEM's ultrathin sections (50-90 nm) cut by glass knives, SEM uses diamond knives for thicker sections or whole mounts.
- Heavy metal coatings provide conductivity for electrons.
- Electrons are reflected from the specimen.
- Electrons scan the surface of the specimen.
- Gives 3D-like images of surfaces – pollen grains, insect legs, microchips.
- Resolution can be around 1 – 20 nm.

Figure 9.6: A. Transmission Electron Microscope B. Scanning Electron Microscope

(Source: Wikimedia commons)

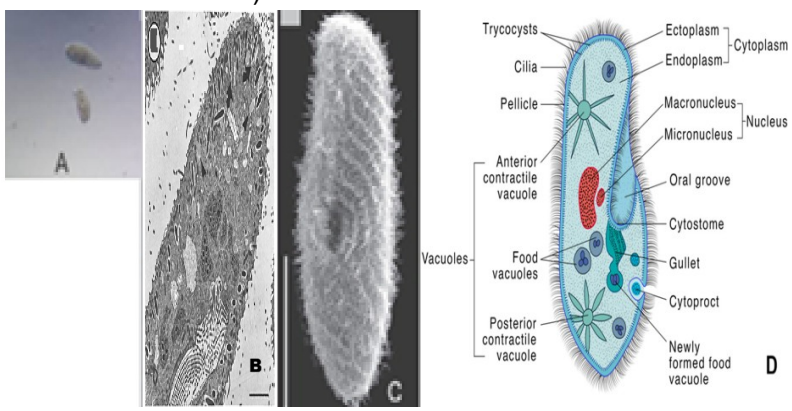


Figure 9.7: *Paramecium* as observed under - A. Light Microscope (Wikimedia commons); B. Transmission electron microscope (Source: Research Gate); C. Scanning electron microscope (Source: Research Gate) and D. Diagrammatic representation.

9.5.3.3 Let us compare Light microscope and Electron microscope (TEM and SEM)

The comparison between these three types of microscopes can be summarised as below:

S. No.	Feature	**Light Microscope**	**Transmission Electron Microscope**	**Scanning Electron Microscope**
1.	Illumination source	Visible light	Electron beam (broad)	Electron beam (focused, scanned)
2.	Types of lenses	Glass (convex, achromatic)	Electromagnetic coils (condenser, objective, projector)	Electromagnetic coils (condenser, scanning, objective)
3.	Thickness of section	Up to several mm - whole mounts; 5 - 10 µm for tissue sections	Ultra-thin (<100 nm, typically 50-90 nm ultramicrotomy)	Surface only (no sectioning; samples 20 - 30 mm thick, coating 10 -100 nm)
4.	Staining	Basic dyes (e.g., methylene blue, eosin; light-absorbing)	Dense metal compounds, such as uranyl acetate and lead citrate.	Conductive coating (e.g., gold/ palladium; no traditional staining)
5.	Observing living cells	Yes (e.g., pond life, cheek cells)	No (vacuum kills cells)	No (vacuum and the coating kills cells)
6.	Resolution	~0.2 µm	~0.1 nm or better	~1-10 nm
7.	Magnification	Up to 1,500x	Up to 50 million x	Up to 2 million x
8.	Sample	Minutes	Hours-days	Hours (dehydration,

	preparation time	(simple mounting)	(embedding, ultramicrotomy)	coating)
9.	Cost	Low (~₹10,000-50,000)	Very high (₹50 lakh+)	High (₹20-50 lakh)
10.	Vacuum required	No	Yes	Yes

Think

Why do you think electron microscopes are usually found in big research centres and not in normal school laboratories?

9.6. What is new in Microscopy? What are the limits?

9.6.1 New Developments

With advances in science and technology, digital microscopes are being developed that show a real time image directly on a screen. Thus, live images can be shared with all students at once. In Super-resolution microscopes details smaller than the normal limits of light can be observed. Explore these latest inventions through books, trusted websites, science magazines, and virtual lab simulations, and find out what new things could we discover by using an even better microscope! Your curiosity today may help design the microscopes of tomorrow.

9.6.2 Limitations

- With a light microscope, structures smaller than about 0.2 µm cannot be resolved due to the diffraction limit of light.
- Electron microscopes are costly, need vacuum and very careful sample preparation; most samples have to be processed with chemicals and metal stains.

9.7. Where do we use Microscopes?

You might be surprised how often microscopes quietly support our lives.

- **Hospitals and Pathology laboratories:** Diagnosing diseases by checking blood, sputum, tissue biopsies (e.g., malaria parasites in blood).
- **Science laboratories:** Studying stomata, plant and animal tissues, plant diseases etc.
- **Industry:** Checking quality of metals, plastics, electronic chips using light and electron microscopes.
- **Police and forensics:** Examining fibres, hair, glass fragments, blood stains from crime scenes.
- **Environment:** Checking water samples for algae, protozoa and pollution indicators.

Snapshots

- Microscope (*micro* – small; + *skopion* - "means of viewing") is a special instrument required to observe tiny living organisms or their parts which cannot be seen through naked eyes.
- Different microscopes have varied magnification powers and resolution ranges; the size of the specimen and purpose of observation determines which microscope can be used to view the specimen effectively.
- Magnification is the factor by which a microscope enlarges a specimen's image relative to its actual size (e.g., 400 X).
- Resolution is the smallest distance between two very close points in a specimen to clearly distinguish them as separate and not merged into a single image. It is often expressed as a unit of length e.g. 0.2 µm for light microscopes.
- Light (Optical) microscopes rely on visible light and glass lenses to enlarge and view specimens.
- Light microscopes function by using refraction—the bending of light as it moves between different media due to changes in speed—along with reflection to direct and focus light rays.
- They can be broadly grouped according to the number of lenses used: simple microscopes have a single lens, while compound microscopes contain two or more lenses.
- Fluorescence microscopy uses high-intensity light to excite specialized dyes in a specimen, causing specific cellular structures to glow brilliantly against a dark background like stars in the night sky.
- Phase-contrast microscopy is a premier technique for observing living cells in their native state because it enhances contrast without requiring chemical stains that would compromise cell viability.
- For light microscopy, we can prepare temporary or permanent mount depending upon the short term or long-term storage requirement.
- Electron microscopes use electron beams (shorter wavelength ~0.005 nm *vs.* light 550 nm) accelerated in vacuum, focused by magnetic lenses.
- Electron microscopes are classified as transmission (TEM) or scanning (SEM) based on how the electron beam interacts with the specimen—either passing through it or being reflected from its surface.
- TEM shows internal details of cells in 2D – mitochondria, ribosomes, viruses etc while SEM gives 3D-like images of surfaces.
- In addition to the study of cell and its organelles, microscopes are used for diagnosing diseases, for assessing the quality of metals, plastics, electronic chips, analysis of water samples and in forensics etc.

Check Your Understanding

1. A microscope has a 10X eyepiece and a 40X objective.
   a) What is its total magnification?
   b) At this setting, the field of view is 0.4 mm. If 4 cells fit across, estimate the size of one cell.
2. a) You want to watch live protozoa moving in pond water. Which microscope (light, phase-contrast, TEM, SEM) is best and why?

   b) Neha wants to study the 3D surface of a pollen grain. Which microscope should she choose and why?
3. Riya sees a sharp onion cell image at 100X, but when she switches to 400X, the image is big but very blurred. Name the concept causing this problem. Explain the reason.
4. Draw a ray diagram of a compound microscope.
5. Design a simple poster "How to take care of a microscope?" with three do's and three don'ts.
6. At 40X total magnification, the field diameter is 4 mm. Predict the field diameter at 400X magnification (assume it is inversely proportional to magnification).
7. A student accidentally traps many air bubbles while placing the cover slip. How will this affect observation? Suggest two ways to avoid bubbles next time.
8. Compare TEM and SEM in terms of:
   - Type of image.
   - Best use (internal *vs* surface).
9. Plan a brief investigation using a school light microscope to compare the purity of three water samples (tap water, RO-purified water, and pond water). Outline the main steps and predict your expected observations.
10. Can we rely on electron microscopes for studying living cells? Explain the reason.
11. List two ways how microscopes are used in hospitals and one way they are used in industries that manufacture mobile phones.
12. Imagine you are Robert Hooke. Write a 5–6 lines diary entry about what you felt when you first saw "little boxes" (cells) in cork.
13. Ananya says, "If we add more and more lenses, we can see anything, even atoms, with a school microscope." Use the idea of resolution to correct this statement.

14. Nishant wants to observe the effect of concentrated salt solution on cells of *Rhoeo* leaf and also wants to keep slides for future reference. Answer the following:
    a) Which type of mount should be used for this purpose? Give reason. b)
    b) Will the same slide be suitable for long-term storage? Elucidate the reason.
15. Why is it important to fix and dehydrate cheek cells before mounting in Canada Balsam for school laboratory storage? Predict the consequences if a student inadvertently skipped the fixation and dehydration steps before mounting the specimen in Canada Balsam.

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