

CHAPTER ONE

MICROSCOPY

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The light microscope is a precision instrument that enables the user to see the order and organization inside a very small living structure. It plays an important role in understanding the compound picture of the structure(s) and function(s) of living material. For effective use of the microscope, it must be aligned correctly, handled carefully and kept clean at all times.

With regards to the microscope, the students should know and be able to do the following:

- Know the different components of the microscope;
- Understand the function of each component of the microscope;
- Insert and move a slide on the stage;
- Adjustment of the lighting mechanism of the microscope;
- Adjust the microscope to your eyes, including focus and inter-ocular distance;
- Recognise and understand different magnifications of an image;
- Understand and explain the pathway of light through the microscope; and
- Recognise and understand the different colours and shades on a slide.

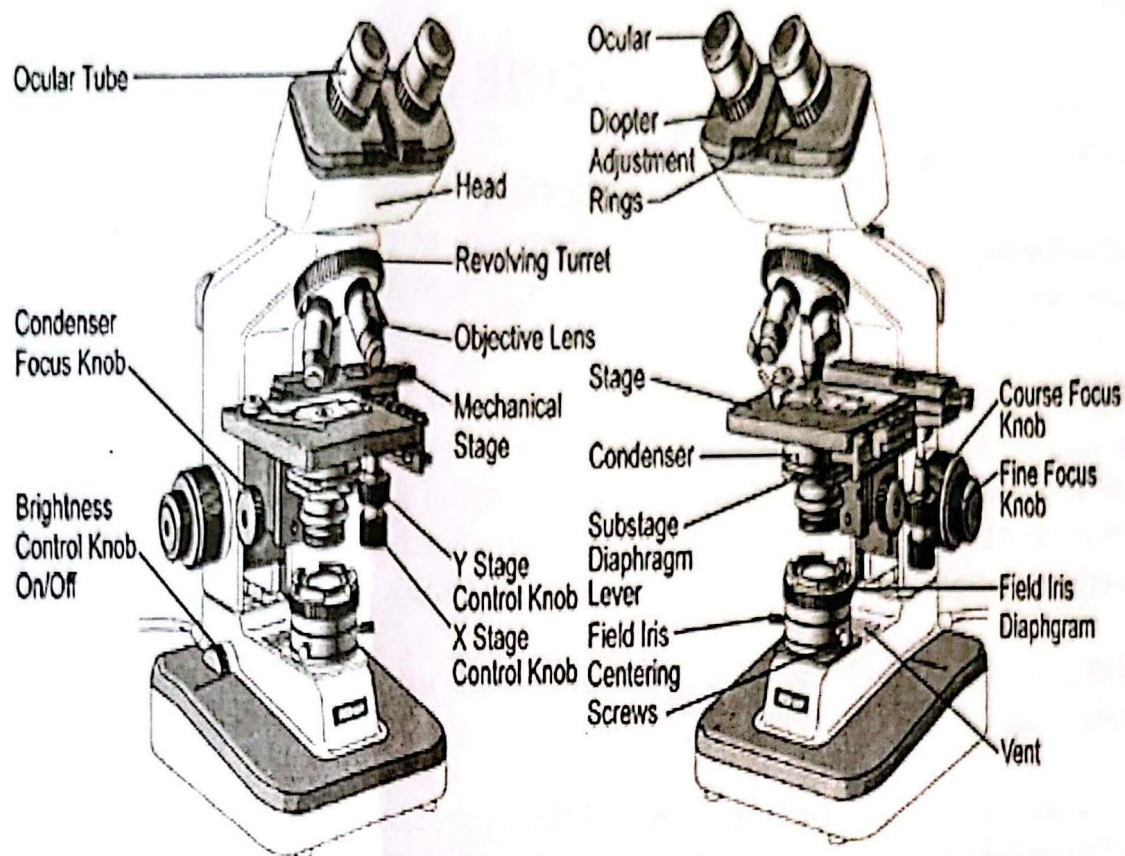


Figure 1: Anatomy of a Light Microscope.

1.1. Identification and Functions of the Component Parts of a Microscope (Figure 1).

The microscope comprises the following:

- (a) Microscope Stand, which is made up of:
 - (i) Frame
 - (ii) Coarse and fine focus knobs
 - (iii) Light source
 - (iv) On/Off power switch
 - (v) Variable light control
- (b) Integrated Binocular Tube, which consists of:
 - (i) Eyepiece
 - (ii) Revolving Nosepiece carrying the objectives of various magnifications:
 - (a) Very low magnification 4X
 - (b) Low magnification 10X
 - (c) High magnification 40X
 - (d) Oil immersion 100X

- (c) **Mechanical Stage**, which is made up of:
 - (i) Specimen holder with spring-loaded clamps and Control Knobs for movement of the specimen.
 - (ii) Condenser.

Each part of the microscope has the following functions:

Frame

The various parts of the microscope are mounted on the frame.

Coarse and Fine Focus Knobs

Adjustment wheels move either the stage or the microscope tube to focus the image seen at the ocular lens. At higher magnifications, only the fine focus knob is used.

Light Source

This provides illumination of the specimen.

On/Off Power Switch

Turns power to the light source on or off.

Variable Light Control

The variable light control adjusts the intensity of the illumination.

The Mechanical Stage

The stage below the objective lenses with a spring loaded clamp arrangement to support and move the slide on the stage.

Condenser

The condenser focuses light from the source(s) in a cone onto the tissue. It usually incorporates diaphragms and filters to manage both quality and intensity of illumination.

Revolving Nose Piece

The revolving nose piece or objective turret carries the objective lens and enables the user to easily change to a higher or lower magnification.

Objective Lenses

Usually but not restricted to 4X, 10X, 40X and 100X (oil immersion) magnification. The objective lenses receive light from the tissue on the slide and project an enlarged image in the direction of the ocular lens. Some types of objective lenses use oil or water placed on top the specimen or coverslip to obtain greater resolution at higher magnifications.

Ocular or Eyepiece Lens

The eyepiece or ocular lenses further magnified and enhance the image from the objective lens and brings the image into focus for the eye. The final optical magnification of the specimen is the product of the magnification of the ocular and objective lenses. The eyepiece often contains a diopter adjustment ring which can be used to adjust the focus for the user's own eyes.

Lens Structure and Focal Point

Objective lenses of microscopes are made of special optical glasses with higher quality than ordinary glasses. The lens is shaped like a circular disk with two outward curving convex faces. When parallel rays of light strike one face of the objective lens, they are focused while passing through the lens to meet at a single spot called the Focal Point.

Focal Length of an Objective Lens

The focal length of an objective lens is the distance from the centre of the lens to the focal point. It is the distance from the lens to the point of convergence of parallel rays passing through the lens. The larger created image is, therefore, essentially the object viewed by the ocular- or eyepiece- lens as created by an objective lens with a smaller focal length. The Focal Length of convex lenses usually has a positive sign because the image occurs on the other side of the lens from where the object is positioned. However, Focal Length of concave lenses (where the faces of lens curve inward) is usually negative.

Lens Strength

The strength of a lens is determined by its focal length. The Lens Strength is calculated by taking the inverse of the Focal Length. A lens with a shorter focal length will have a higher lens strength with capacity to

enlarge the image more. Hence, microscopes have shorter focal length so that they can greatly enlarge images.

Calculation of Total Magnification and Focal Length

Total Magnification = Power of the Objective Lens X Power of the Ocular Lens

Magnification (Objective) = Focal Length of Ocular Lens / Focal Length of the Objective Lens

1.2. *How to use the Light Microscope*

This is a very condensed version of the correct way to use a light microscope. Consult a manual or textbook if you plan to do serious work using a microscope.

However, you are expected to be able to:

- Focus and view slides effectively
 - Know what objective lens to be use at a particular time when viewing the slide
 - Know how and when to use the fine and coarse adjustment knob
 - Know how to calculate the magnification of the specimen.
- (a) Microscope quick checklist:
1. Condenser at the top
 2. Iris should be opened
 3. Focus with both eyes or one eye depending on the number of eye piece available
 4. Rheostat supply enough light
- (b) Using the microscope:
1. Handle the microscope by the stand only
 2. Remove the microscope from its box beneath the table and place it on the desk
 3. Ensure that there are no wires, books or pens under the base.
 4. Turn the rheostat to 5
 5. Open the iris on the condenser all the way
 6. Turn the condenser as low as it can go
 7. Open the spring-loaded finger of the specimen holder and insert the slide. The specimen should be above the condenser in the path of the light. Ensure that the cover slip faces up

8. Switch on the microscope
9. Turn the condenser directly beneath the slide
10. Adjust your chair until the level of your eyes is slightly higher than the level of the oculars
11. Look through any of the two oculars and adjust the light (using the rheostat) to a comfortable level (5-8)
12. Move the oculars apart
13. Position your eyes approximately 2cm from the oculars
14. Slowly move the oculars closer to each other until you see two circles or two half circles
15. Move the oculars slowly until you see only one circle
16. Close your right eye. Your left eye must see an image. If the right ocular has a pointer, it should not be visible
17. Open both eyes. You should see one image with both eyes. If the ocular has a pointer, it should be visible with both eyes
18. If you see one image with both eyes, you can start focusing the image.
19. Ensure that the 4x objective is in position above the slide. This can be done by turning the nosepiece until the desired objective lens is in position
20. Close your left eye
21. Turn the coarse focus adjustment knob and focus the image in the right ocular
22. Adjust the right image by turning the fine focus knob
23. Close your right eye
24. Focus the right image by turning the diopter adjustment ring
25. Open both eyes and focus the image by turning the fine focus knob After this it is not necessary to turn the diopter adjustment ring
26. Turn the nosepiece until the 10x objective clicks into place above the condenser
27. Focus the image only with fine focus knob
28. Turn the nosepiece until the 40x objective clicks into place above the condenser
29. Focus the image only with fine focus knob
30. Turn the nosepiece until the 10x objective clicks into place above the condenser

31. Focus the image only with fine focus knob
32. Turn the nosepiece until the 40x objective clicks into place above the condenser
33. Focus the image with the fine focus knob

Questions:

1. Calculate the highest total magnification for a microscope with an ocular lens of 15X and objective lenses of 5X, 10X, and 30X.
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2. If linear magnification of the objective lens of a microscope is 30, angular magnification of the ocular lens is 55 and the focal length of the ocular lens is 15cm. Calculate the focal length of the objective lens.
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1.3. Identification of the Structural Components of the Cell

Slide 1: This specimen (Amphibian Blood) serves as an example of an animal cell and is used for orientation regarding the size, shape, colour and shading of a cell stained with haematoxylin and eosin. Red blood cells (erythrocytes), are anucleated biconcave discs, which are packed with the O₂-carrying protein hemoglobin. The biconcave shape provides erythrocytes with a large surface-to-volume ratio to ease gas exchange. Human erythrocytes are 7.5µm in diameter, 2.6µm thick at the rim, and 0.8µm thick in the center when suspended in an isotonic medium. The normal concentration of erythrocytes in blood is approximately 3.9–5.5 million per microliter in women and 4.1–6 million per microliter in men.

At the end of this section, you should be able to identify:

- The red blood cell
- The cell membrane
- The shape of the cell and its nucleus
- The location of the nucleus in the cell i.e. central or eccentric

- The ratio of the size of the nucleus to the size of the cell
- The colour of the nucleus
- The colour of the cytoplasm
- Any other organelle within the cytoplasm of the cell
- Any granule within the cell

Drawing:

Draw a red blood cell: Draw the shape and size of the cell and nucleus in the correct ratio to one another.

Questions:

1. What is understood by the terms acidophilic and basophilic?

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2. Complete the table:

	Cytoplasm	Nucleus	Cell membrane
Colour			
Stain			
Visible			

3. Can you see the cell membrane? If yes, describe the cell membrane. If no, give reasons.

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4. Can you locate the nucleus of the cell? If no, explain.

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5. The average diameter of the amphibian red blood cell is 0.01mm. How many red blood cells would be equal to the average diameter of a human red blood cell?

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6. Which is the largest human cell? Which is the longest human cell?

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CHAPTER TWO

EPITHELIAL CELLS

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These are cells that line the inner surfaces of cavities of internal organs as well as the outer surfaces of organs and blood vessels. At the end of this section, the students are expected to be able to identify the different kinds of epithelial cells present in different body tissues based on the following:

- * The type, size and form or shape of cell present i.e. squamous, cuboidal, columnar e.t.c.
- * The number of cell layers i.e simple or stratified.
- * Cell borders – visible or not.
- * Position of the cells in relation to each other and the underlying basal lamina.
- * Nature of the free or luminal surface i.e. ciliated, brush border etc.
- * Presence of glands and hair.
- * Types of glands present e.g. single cell, simple, tubular etc.
- * The position of the nucleus in each of the cell.

2.1 Lining Surface Epithelial Cells

The aim of this practical session is to illustrate epithelial tissue. The type, cell form, examples of distribution and functions of lining epithelial cells are provided below.

Type of Epithelial Cells	Cell Form or Shape	Examples of Distribution	Main Function
Simple	Squamous	Lining of vessels (endothelium).	Facilitates the movement of the viscera (mesothelium), active transport by pinocytosis (mesothelium and endothelium), secretion of biologically active molecules (mesothelium).
		Serous lining of cavities; pericardium, pleura, peritoneum (mesothelium).	

Type of Epithelial Cells	Cell Form or Shape	Examples of Distribution	Main Function
	Cuboidal	Covering the ovary, thyroid.	Covering, secretion.
	Columnar	Lining of intestine, gallbladder.	Protection, lubrication, absorption, secretion.
Pseudostratified	Some columnar and some cuboidal	Lining of trachea, bronchi, nasal cavity.	Protection, secretion; cilia-mediated transport of particles trapped in mucus.
Stratified	Surface layer squamous keratinized (dry)	Epidermis.	Protection; prevents water loss.
	Surface layer squamous nonkeratinized (moist)	Mouth, esophagus, larynx, vagina, anal canal.	Protection, secretion; prevents water loss.
	Cuboidal	Sweat glands, developing ovarian follicles.	Protection, secretion.
	Transitional: domelike to flattened, depending on the functional state of the organ	Bladder, ureters, renal calyces.	Protection, distensibility.
	Columnar	Conjunctiva.	Protection.

Each specimen must be studied as follows:

1. General structure

- (a) The type, size and form or shape of cell present i.e. squamous, cuboidal, columnar e.t.c.
- (b) The number of cell layers, i.e. squamous or stratified.
- (c) Cell borders – visible or not.
- (d) Position of the cells in relation to each other and the underlying basal lamina.
- (e) Nature of the free or luminal surface e.g ciliated, brush border etc.
- (f) Presence of glands and hair.
- (g) Types of glands present e.g. single cell, simple, tubular e.t.c.
- (h) The position of the nucleus in each of the cell.

2. Cell structure

a) Nucleus

- (i) Staining reaction and degree of granularity of chromatin.
- (ii) Position in the cell.
- (iii) Shape and size.
- (iv) Shape and size in comparison to the shape and size of the cell.

b) Cytoplasm

- (i) General appearance and texture.
- (ii) Staining reaction
- (iii) Presence of inclusions such as prominent granules, vacuoles, pigments etc.

Questions:

1. How are the functions of epithelial cells adapted to their features?

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2. How will you systematically approach identification of the type of epithelium on a slide?

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2.1.1. *Simple Squamous Epithelium (Mesothelium)*

Slide 2: This is a spread preparation of the mesenterium. The simple squamous epithelium is a thin serous membrane made up of only one cell layer such that every cell is in direct contact with the underlying basement membrane. It can be found in the peritoneal cavity of the abdomen, pleura and other flat surfaces where passive diffusion of gases occur. The mesenterium has two outer layers of mesothelium and an inner core of connective tissue. Because the specimen is a spread of the membrane on the slide and not a sectioned piece of tissue, the mesothelium is studied looking at the surface of the cells. This slide serves as an example of cells organized into a continuous layer with the cell borders clearly visible. By focusing up and down both mesothelial layers can be seen.

Identify:

- (i) Cell borders
- (ii) Shape of the cells.
- (iii) Large lightly stained nuclei.

Note: Connective tissue cell nuclei are discussed later under connective tissue.

Draw and annotate:

A surface view of a few cells and their nuclei indicating the shape of the cells by means of their borders:

Questions:-

1. How do the cell borders appear?

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2. How do we know these cells are thing?

.....

2.1.2. Simple Squamous Epithelium (Endothelium)

Slide 3: This specimen serves as an example of endothelium as seen in a section. Examples of specimen used here are sections through the section of the ileum. The specimens that are used are sections through the skin of the palm or a section through the ileum can also be used. The blood vessels can be found in the dermis/hypodermis of the skin or in the submucosa of the ileum. Simple squamous epithelium is a single thin layer of squamous cells that lines the interior surface of blood vessels and lymphatic vessels thereby forming an interphase between the circulating blood or lymph within the lumen.

Identify:

Macroscopic

Hold the slide against a white background and look at it with the naked eye. Identify the three coloured layers seen in the section:

- (i) A dark pink to red scalloped area – the epidermis.
- (ii) A central light pink area – dermis.
- (iii) A thick very light pink area – the hypodermis.

The blood vessels can be found under low magnification in the dermis.

Microscopic

- (i) Lumen of the blood vessel
- (ii) Endothelial cells against the lumen
- (iii) Nuclei of the endothelial cells
- (iv) Cytoplasm
- (v) Layers of the blood vessel wall

Draw and annotate:

A line diagram of the blood vessel and endothelial cells.

Questions:

1. What is characteristic of the endothelial cells?
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2. What is characteristic of the cytoplasm of these endothelial cells?
And the nuclei? What are characteristics of the cytoplasm and
nuclei of these endothelial cells?
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.....
3. What is sometimes seen in the lumen?
.....

2.1.3. Simple Cuboidal Epithelium

Slide 4: This specimen is a section through the kidney.

Identify:

Macroscopic

- (i) The mass of tissue forming the kidney.
- (ii) The cortical and medullary areas of the kidney.

Microscopic

- (i) Simple cuboidal epithelium that lines certain of the tubules. These cells are as wide as they are high.

Draw and annotate:

A few simple cuboidal epithelial cells lining the tubules. Also, indicate the connective tissue that these cells rest on.

Questions:

1. Describe the shape of these cells.
.....
2. Are the cell borders of these cells visible?
.....
3. How is the width of these cells determined?
.....
4. Why does the simple cuboidal epithelium appear stratified in certain places?
.....

2.1.4. Simple Columnar Epithelium

Slide 5: This specimen is a section through the gallbladder.

Identify:

Microscopic

- (i) Lumen of the gallbladder
- (ii) Simple columnar epithelial cells

Draw and annotate:

Simple columnar epithelial cells. Also indicate the connective tissue layer that these cells rest on.

Questions:

1. How are the lateral borders of the cells determined?
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.....
2. Where are the nuclei located and what is their shape?
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3. What is the appearance of the free surface of these cells? What is absent from the free surface of these cells?
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2.1.5. Simple Ciliated Columnar Epithelium

Slide 6: This specimen is a section through the uterine tube.

Identify:

Microscopic

- (i) Lumen of the uterine tube.
- (ii) Ciliated as well as non-ciliated columnar epithelial cells

Draw and annotate:

A few simple ciliated columnar epithelial cells. Indicate the cilia and basal bodies clearly, as well as the connective tissue that the cells rest on.

Questions:

1. What is present on the free surface of these cells?
.....
2. How do the cilia and basal bodies appear microscopically? What are the microscopic features of the cilia and basal bodies?
.....
.....
.....
3. How does the shape of these columnar epithelial cells compare to those of the gallbladder?
.....
.....

2.1.6. Pseudostratified Ciliated Columnar Epithelium

Slide 7: This specimen is a section through the trachea.

Identify:

Macroscopic

- (i) The ring shaped trachea.
- (ii) The horseshoe shaped hyaline cartilage.

Microscopic

- (i) Lumen
- (ii) The four types of cells that are found in this epithelium.

Draw and annotate:

A few epithelial cells in which all four types of cells are present. Clearly indicate each of the four cell types.

Questions:

1. How does each one of the four cell types appear in this epithelium?

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2. Why is this epithelium described as pseudostratified?

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3. What are the functions of each of the four cell types?

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2.1.7. Thin Stratified Squamous Non-keratinized Epithelium

Slide 8: This specimen is a section through the eye. The thin stratified squamous non-keratinized epithelium is found on the outside of the cornea. Stratified epithelia occur where there are high levels of friction.

Identify:

Macroscopic

- (i) The eyeball and the cornea situated interiorly.

Microscopic

- (i) The different layers comprising the epithelium.
- (ii) The shape of the cells in the different layers.

Draw and annotate:

A few epithelial cells of each layer showing the different cell shapes.

Questions:

1. How many cell layers form this epithelium and how thick is each one of these layers in relation to one another?

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2. How does the shape of the cells in each layer change?

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3. What are the characteristics of the junction between this epithelium and the underlying support structures (connective tissue)? Are papillae present?

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2.1.8. *Thick Stratified squamous Non-keratinized Epithelium*

Slide 9: This specimen is a section through the oesophagus.

Identify:

Macroscopic

- (i) The circular oesophagus.
- (ii) The lumen

Microscopic

- (i) The different layers of the epithelium
- (ii) The shape of the cells in the different layers

Draw and annotate:

A few epithelial cells of each layer to show the different cell shapes.

Questions:

1. How many cell layers are present between the basal lamina and lumen?
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2. How thick is each one of the different layers in relation to one another?
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.....
3. How does the shape of the cells in each layer change?
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.....
4. What is characteristic of the junction between this epithelium and the underlying support structures (connective tissue)? Are connective tissue papillae present?
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.....
5. How does this junction appear in longitudinal as well as cross section?
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2.1.9. Stratified Squamous Keratinizing Epithelium

Slide 10: This specimen is a section through the skin of the palm of the hand.

Identify:

Macroscopic

Hold the slide against a white background and look at it with the naked eye. Identify three coloured layers seen in the section:

- (i) A dark pink to red scalloped area – the epidermis
- (ii) A central light pink area – the dermis
- (iii) A thick but very light pink area – the hypodermis

Microscopic

- (i) The different layers of the epithelium which form the epidermis
- (ii) The shape of the cells in the different layers.

Draw and annotate:

A few epithelial cells of each layer to show the different cell shapes.

Questions:

1. How many cell layers are present?

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2. How does the shape of the cells in each layer change?

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3. How does the junction between this epithelium and the underlying support structures (connective tissue) appear?

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2.1.10. Urothelium (Transitional)

Slide 11: This specimen is a cross section through the ureter.

Identify:

Macroscopic

(i) The circular ureter

(ii) The lumen

Microscopic

(i) The different layers of the epithelium

- (ii) The shape of the cells in the different layers

Draw and annotate:

A few epithelial cells to show the different cell shapes.

Questions:

1. How many layers can be identified in the epithelial cells you have drawn?

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2. How does the shape of the cells in each layer change? What is characteristic of the luminal layer? Why?

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Revision Notes on Lining Surface Epithelial Cells:

1. Name the 3 main cell types, according to shape.

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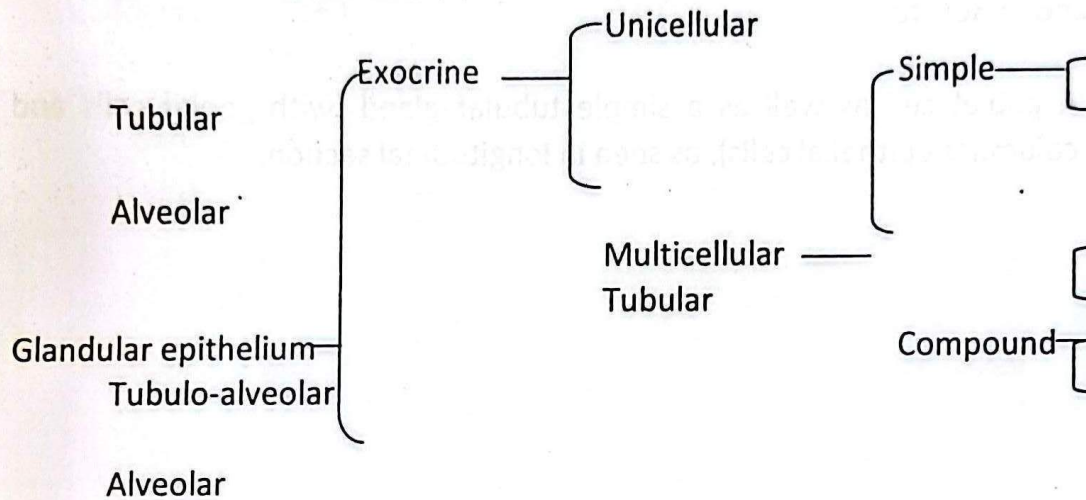
2. What is the difference between stratified and pseudostratified epithelium?

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2.2 Specialized or Glandular *Epithelial Cells*

The Specialized or Glandular epithelial cells will be studied according to the following scheme:



At the end of this sections, students are expected to know the concept of double glands i.e. pancreas, testes, ovary which have the ability to secrete both endo- and exocrine substances.

2.2.1. *Goblet Cells and Simple Tubular Glands*

Slide 12: This specimen is a longitudinal section through the large intestine.

Identify:

Macroscopic

- (i) The longitudinally sectioned colon
- (ii) The luminal surface

Microscopic

- (i) Single cell gland or goblet cell (pale staining)

- (ii) Simple tubular gland. Note that this gland is the whole test tube shaped structure and that it is lined with goblet cells and columnar epithelial cells.

Draw and annotate:

A goblet cell as well as a simple tubular gland (with goblet cells and columnar epithelial cells), as seen in longitudinal section.

Questions:

1. How are the functions of Glandular epithelial cells adapted to their features?

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.....

2. What are the characteristics of a goblet cell?

.....

3. What cells form a simple tubular gland?

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2.2.2. Compound Tubulo-alveolar gland

Slide 13: This specimen is a section through the pancreas.

Identify:

Macroscopic

- (i) The mass of pancreas tissue

Microscopic

- (i) The round or elongated secretory units. These are groups of cells arranged around a central lumen.

Draw and annotate:

A line diagram of the secretory units in longitudinal, cross, as well as oblique sections. In the line drawing of the section also draw the cells forming the unit.

Questions:

1. What are the characteristics of these glandular cells?

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2. Why do the secretory units have different shapes in a section?

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2.2.3. Secretory Units

Slides 14 and 15: These specimen are sections through the pharyngeal tube and the tip of the tongue respectively, which are used as examples to study serous, mucinous and mixed secretory units.

Identify:

Macroscopic

Identify with the naked eye the J-shaped cartilage of the pharyngeal tube. The glandular tissue can be found on the inside of the J-shaped cartilage. The glandular tissue of the tongue can be found between the bundles of muscle fibers.

Microscopic

- (i) Serous units
- (ii) Mucinous units
- (iii) Mixed units – serous demilunes

Draw and annotate:

The three types of units. Clearly indicate the differences in morphology.

Questions:

1. Complete the following table to illustrate the differences between mucinous and serous units.

	Mucinous	Serous
Shape of unit		
Staining of cells in unit		
Shape of nucleus		
Lumen		

2. Where do the secretions of the serous demilunes go?

.....
.....

2.2.4. Islands

Slide 16: This specimen is a section through the pancreas to demonstrate the organization of endocrine glands.

Identify:

- (i) The mass of pancreas tissue

Macroscopic

- (i) An island among the exocrine glandular tissue
Look at the following slides to see the different ways cells in endocrine glands are organised:

Groups	-	hypophysis	-
Cords	-	adrenals	-
Follicles	-	thyroid	-

Draw and annotate:

A few cells of an island. Demonstrate the cellular arrangement in the island clearly.

Additionally, draw a few (generic) cells to demonstrate the shape of groups, cords and follicles.

Revision Notes on Specialized or Glandular Epithelial Cells:

1. Name and define the 4 types of epithelial secretions.

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2. Describe with examples, the mode of distribution of glands of specialized epithelial cells.

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3. Name an example of tumours of glandular epithelial cells.

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