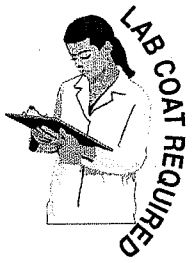
CELLS & EPITHELIUM



Laboratory Objectives

On completion of the activities in this laboratory session, you will be able to:

- Correctly carry, use and care a bright field light microscope.
- Calculate the total magnification of the lenses that you are using to view an object.
- Describe the principle of inversion of an image by the microscope.
- Prepare and observe a cheek epithelial cell smear.
- Demonstrate strategies for examining light and electron micrographs.
- Understand the unique properties of epithelia and how these relate to the tissue's function.
- Recognise different types of epithelial tissue in histological sections.

Materials

A laboratory coat is required for Activity 3.2.

Each pair of students should obtain a microscope, slide box, and a slide marked with the letter "e". Additional material will need to be collected from the font of the laboratory for Activity 3.2.

Preliminary Reading

Tortora GJ and Derrickson B 2006 Principles of Anatomy and Physiology 11th Ed. Wiley, New York. Chapters 3 and 4.

The Light Microscope

Human cells are far too small to be viewed unaided and therefore must be observed with a microscope. Among the smallest cells in the human body are red blood cells which average 7 micrometers (μm) in diameter. (1 micrometer = 1/1000 of a millimetre).

In today's laboratory session you will be required to learn how to use an Olympus CH model light microscope (Figures 3.1.a/b). Please carefully read the following instructions on the proper handling and use of this microscope before starting Activity 3.1. Consult a demonstrator if you require further assistance.

HANDLING OF THE MICROSCOPE

The microscopes you will be using this year to study microscopic anatomy are expensive, precision instruments. They can be damaged if they are not handled with care. You will be charged for any repairs to the microscopes if damage was found to be the result of careless handling. Adherence to the following rules will prevent any damage to the microscopes:

- Always carry the microscope with two hands in the upright position. Grasp the neck (arm) with one hand, and support underneath the base with the other hand.
- Do not touch the lenses with your fingers. If you accidentally do so, clean the lens immediately with a lens cleaning tissue (ask a demonstrator for this tissue).

USE OF THE MICROSCOPE

The following instructions must be followed whenever you are required to use a microscope. Please read these instructions carefully before commencing Activity 3.1.

1. Collect a microscope and slide box from the microscope cupboard. You may need to share these with your laboratory partner.

2. Set the microscope on the bench in with the eyepiece pointing towards you. BEFORE connecting and switching on the power, check that the light intensity is set at zero.
3. Plug in and switch on the power.
4. Open the iris diaphragm of the condenser fully.
5. Rotate the 4x (lowest magnification) objective lens into position using the revolving nosepiece.
6. Use the course adjustment knob to move the microscope stage down to make room for placing a slide on the specimen stage.
7. Place a slide on the microscope stage. Secure the slide in position with the slide holder.
8. Bring the specimen into the pool of light using the two stage control (coaxial) knobs.
9. Raise the microscope stage so that it is close to the objective lens.
10. Look into the microscope through the eyepiece lenses. Gradually manually move the eyepiece lenses either closer together or farther apart until the mages from both eyes fuse into one circular image.
11. Adjust the iris diaphragm so that an adequate amount of light passes through the tissue section.
12. Focus the image by slowly turning the coarse adjustment knob. When the image is approximately in focus, is the fine adjustment knob to bring it to exact focus.
13. Select an area on the slide that you wish to view at higher magnification using the coaxial stage controls. Rotate the revolving nosepiece so that a higher magnification (first 10X, then 40X) objective lens is in position. Make sure the lens will not hit the slide before moving it into position. DO NOT ATTEMPT TO FOCUS WITH THE COARSE ADJUSTMENT KNOB UNDER HIGH POWER.
14. When you complete your microscope observations:
    - Remove the slide from the microscope stage and return it to the slide box.
    - Set the light intensity back to zero.
    - Rotate the 4X objective lens to the viewing position.
    - Turn off the main switch.
    - Wrap up the power cord and secure it to the neck of the microscope.
    - Return the microscope to the correct numbered position in the cupboard using the two-handed carrying method described earlier.

Figure 3.1.a The Light Microscope, Front View

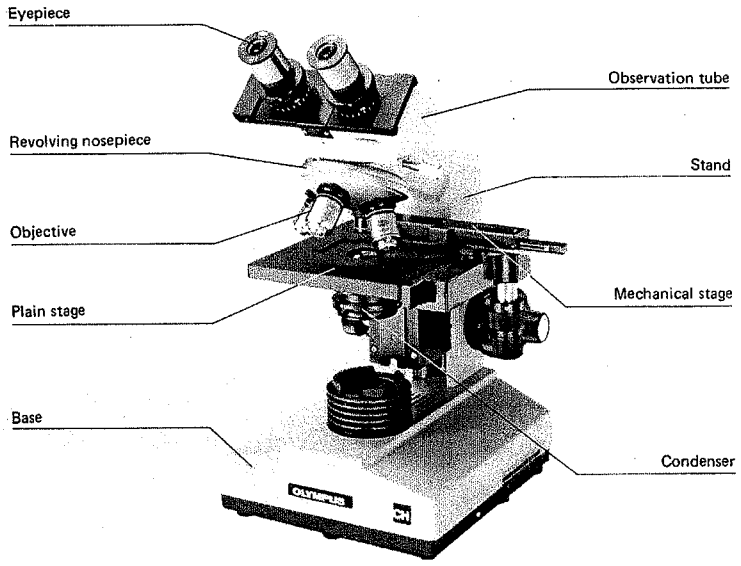
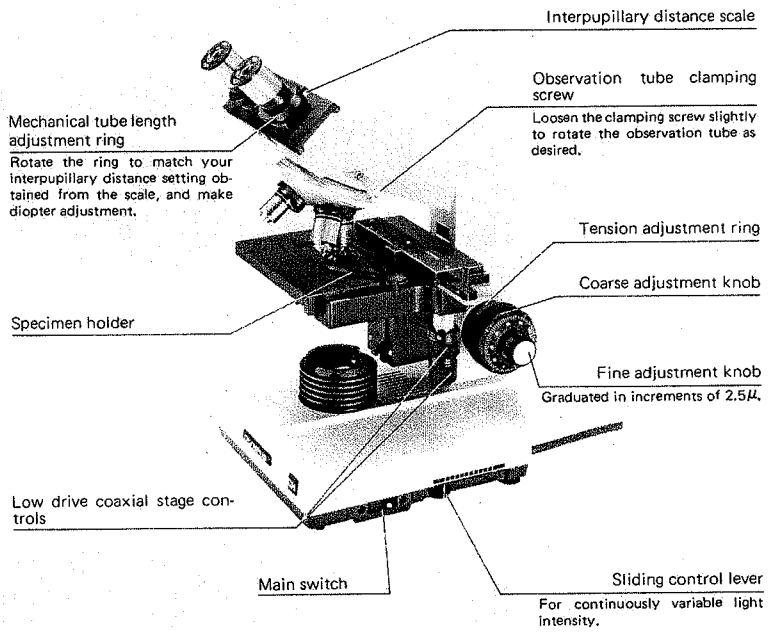


Figure 3.1.b The Light Microscope, Right Side View



ACTIVITY 3.1 Magnification and Alteration of Image When Viewed with the Light Microscope

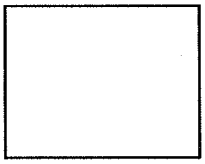
To complete this Activity, you will need to collect a microscope and a slide marked with the letter "e".

1. In the study of microscopic structures, it is important to have an appreciation of the scale (i.e. size) of the objects being studied. This can be achieved by calculating the total magnification of the specimen.

   Total magnification = magnification of objective lens x magnification of eyepiece lens

   a. State the magnification powers of each of the objective and eyepiece lenses.
   Objective lenses: _____Eyepiece lense: _____
   b. Calculate the lowest and highest magnifications possible using your microscope:
   Lowest magnification: _____Highest magnification: _____

2. Place the prepared microscope slide with the letter "e" on the microscope stage in the right side up position and centered over the aperture (i.e. facing you). Place the lowest power (4X) objective lens into viewing position, and observe the letter "e" through the eyepiece lenses. (You may need to focus on the letter "e" using the coarse and fine adjustment knobs).
   a. In the space below, illustrate the "e" as it appears under low power. How is the orientation of the "e" (and any other specimen observed) altered when viewed through the microscope?



_____
_____
_____
_____ **Activity 3.1** continues next page...

Activity 3.1 continued...

b. Use the coaxial stage controls to move the slide to the right. In what direction does the "e" move when viewed through the microscope? _____

c. Use the coaxial stage controls to move the slide away from you. In what direction does the "e" move when viewed through the microscope? _____

3. If you are viewing a tissue section and decide to move from a lower to higher magnification, how will the field of view change? _____

_____

_____

The Cell

The basic structural and functional unit of the human body is the cell.

Each of a cell's parts, or organelles, as well as the entire cell, is organised to perform a specific function. Cells have the ability to grow, reproduce (divide), move, and respond to stimuli. The cells of the body differ in shape, size and specific roles, however, all contain virtually the same basic components. Cells that are similar in structure and function form tissues which, in turn, construct the various body organs.

PARTS OF A CELL

- **Plasma membrane (plasmalemma)** = external membrane which serves as a dynamic interface, and selective barrier, between the internal environment of the cell and its various external environments.
- **Nucleus** = largest structure within a cell. It contains the cell's DNA and directs all cellular activities. The nucleus is bounded by a nuclear envelope, which has numerous opening s called nuclear pores. Suspended in the nucleus is the nucleolus, where ribosomes are made. The appareance of a cell's nucleus provides a wealth of information about the cell, for example the shape of the cell, its cellular activity, and whether or not it is dividing or dying.
- **Cytoplasm** = consists of all the cellular contents between the plasma membrane and the nucleus. It is composed of two components: the cytosol, or fluid portion, and numerous organelles, each 3ith their own characteristic appearance and function.

EXAMINING A CELL

Human cells can only be observed with the aid of a microscope. Earlier in this laboratory session, you were shown how to use to a basic light microscope, which you will be using throughout the year in laboratory sessions to examine human cells and tissues. The useful magnification of a standard light microscope for student use is approximately 1000-1500X. At this magnification, the shape and general appearance of cells can be seen, however if you wish to observe individual organelles, filaments or nuclear components in more detail, an electron microscope is required. These microscopes allow for magnifications of up to 400,000X. Whilst you will not be handling an electron microscope this year (they are very big, very expensive devices), you will be exposed to, and therefore must learn how to interpret, images (referred to as micrographs) of cells taken, not only with the light microscope, but also with the electron microscope.

ACTIVITY 3.2 Light Microscopic Observation of Cells

In this activity, you will be examining, with the light microscope, the cells that line the inside of your cheeks.

METHOD

1. Place a small drop of distilled (purified, clean) water in the centre of a clean slide.
2. GENTLY and lightly scrape the inner lining of your cheek with the broad end of a flat toothpick.
3. Stir the toothpick vigorously in the drop of water on the slide.
4. Cover the drop with a coverslip lowered onto the slide at an angle to avoid forming air bubbles. If there is too much liquid on the slide, blot dry it off at the edge of the coverslip with a paper towel.
5. Observe the slide with the light microscope, first at low (4X) power and then at high (40X) power. In the space below, draw the appearance of your unstained cheek cells:
6. Repeat steps 1-5, this time adding a drop of 10% methylene blue stain to your water-cheek cell suspension.
   In the space below, draw the appearance of your cheek cells after staining.

Appearance of Unstained Cheek Cells	Appearance of Stained Cheek Cells

7. On your drawing, label the nucleus, cytoplasm and cell membrane. Can you identify any additional structures? If so, label these of your drawing.
8. What effect, if any, did staining with methylene blue have on the cells in your preparation?

   _____
   _____
   _____

9. What are the advantages of staining cells (as well as tissues) before viewing under the light microscope? _____
   _____
   _____

Most of the slides you will look at this year are stained not with methylene blue, but rather the two dyes haematoxylin and eosin.

10. What colour is: (a) haematoxylin? _____(b) eosin? _____

11. What cellular components are stained by:
    a. haematoxylin? _____
    b. eosin? _____

ACTIVITY 3.3 Electron Microscopic Observation of Cells

1. Figures 3.2 a,b show the appearance of the same cell when viewed with the (a) light and (b) electron microscope. Figure 3.2.c is a drawing of the same cell as seen under the electron microscope.

   Compare the cell structural detail that you can observe at light microscopic (LM) and electron microscopic (EM) levels by labelling, on these images, as many of the following components as possible:

   nucleus, nucleolus, nuclear envelope, nuclear pores, cytoplasm, plasma membrane, ribosomes, rough endoplasmic reticulum, smooth endoplasmic reticulum mitochondria, Golgi Apparatus, centriole, lysosome.

Fig. 3.2b
EM, ~7,500X

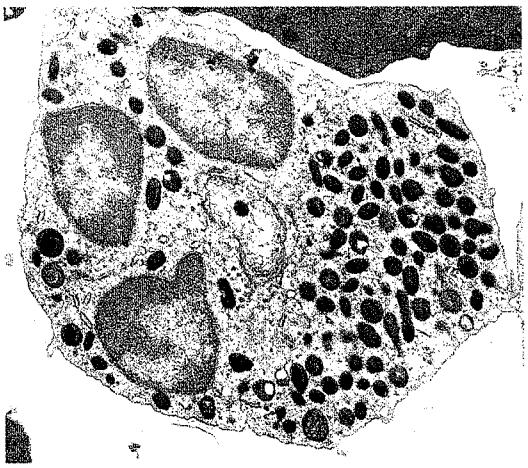


Fig. 3.2a
LM, ~4000X

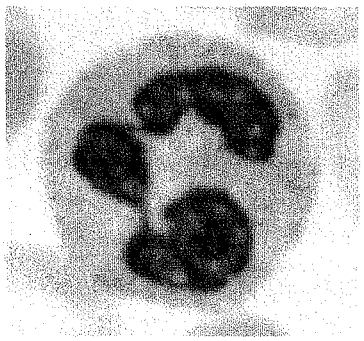
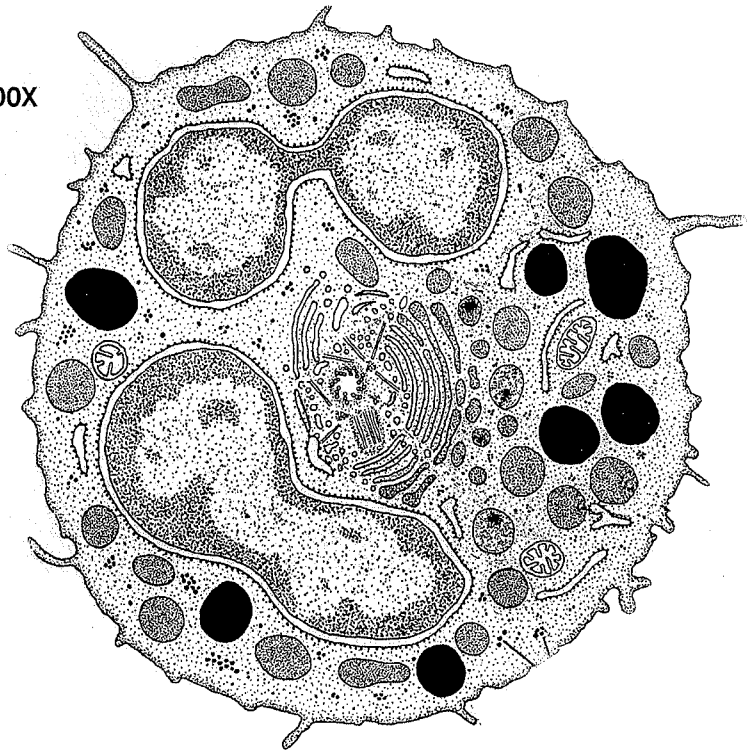


Fig. 3.2c
EM Drawing, ~ 10,000X



EPITHELIUM

TYPES OF TISSUES

A tissue is a group of cells that has a similar embryological origin and works as a unit to carry out a specialised function. The body has four major categories of tissues: epithelium, connective tissue, muscle, and nervous tissue.

EPITHELIAL TISSUE

Epithelial tissue (or epithelium) forms the innermost and outermost surfaces of body structures, as well as the body's glands. Epithelium consists of cells arranged in continuous sheets, in either single or multiple layers. These cells are closely packed together by many junctions.

Epithelium can be classified primarily on the basis of three major characteristics:

1. **Number of cell layers.**
   - Simple epithelium: one layer of cells.
   - Stratified epithelium: more than one layer of cells.
   - Pseudostratfied epithelium: looks multilayered, however not all cells reach surface.
2. **Height and shape of cells.**
   - Squamous epithelium: thin, flat cells.
   - Cuboidal epithelium: square or round cells.
   - Columnar epithelium: tall, narrow cells.
   - Transitional epithelium: cells can change shape from cuboidal to squamous.
3. **Presence of surface modifications** such as cilia, microvilli, keratinisation.

ACTIVITY 3.4 Function and Location of Different Types of Epithelial Tissue

1. The type (i.e. appearance) of epithelial tissue present at any given location in the body is determined by its specific function at that location (i.e. structure correlates to function).
   Match the following epithelial tissues to their descriptions:

   A. Simple cuboidal B. Stratified squamous C. Transitional
   D. Simple columnar E. Simple squamous F. Pseudostratified columnar ciliated

Epithelial Tissue Type	
_____	Lines the eosophagus and forms the skin epidermis
_____	Forms the lining of the stomach and small intestine
_____	Best suited for areas subject to friction
_____	Found in the bladder lining
_____	Lines much of the respiratory tract
_____	Propels substances (e.g. mucus) across its surface
_____	Forms thin membranes; a thin layer of flattened cells

ACTIVITY 3.5 Microscopic Observations Of Epithelial Tissue

For this activity you will require a microscope and slide box.

1. In Activity 3.2 you examined the cells lining the inner surface of your cheek. These cells were, in fact, epithelial cells because they line a body surface.
   a. Obtain Slide 53, Oesophagus and Trachea from the slide box. This slide is stained with haematoxylin and eosin.
   b. Hold this slide up to the light. You will notice two tubular structures. The structure with the more irregularly shaped cavity is the oesophagus. The oesophagus is a hollow tube that connects the mouth to the stomach. View the section of the oesophagus at low power (4X). (Ask a demonstrator if you are unsure which structure is the oesophagus).
   c. You will notice that there are a number of different layers in the wall of the oesophagus. Examine the innermost layer which is the epithelium lining the surface of the oesophagus.
   In the space below, draw the appearance of this layer of epithelial tissue. Make note of the number of layers, and shape and size, of the epithelial cells present, as well as whether or not any epithelial modifications are present.

   Based on your observations and drawing, state the type of epithelium that lines the oesophagus:

   _____

   What is the functional significance of having this type of epithelium lining the oesophagus? _____

   _____

   _____

   _____

   _____

   d. Obtain Slide 27, Fingertip. View the upper margin of the (darker staining portion) of the slide at low power. This region shows the epithelial tissue of the skin, which is called the epidermis. In the space below, draw the appearance of this layer of epithelial tissue.

   What similarities and differences can you see between the epidermis of the skin and the epithelia tissue lining the oesophagus?
   Similarities: _____

   _____

   _____

   Differences: _____

   _____

   _____

   What type of epithelium covers the fingertips? _____