Lab 2. Exercises 4 and 5 - Microscopy and Cell Biology

Overview

This lab will give the student brief explanations of the basic principles by which microscopes work as well as some hands-on experience with the use of the compound microscope, preparation and staining of wet mounts. Students will also learn how to distinguish animal and cell plants viewed under the microscope.

Learning objectives

1. Be familiar with the parts and components of a light microscope.
2. Be familiar with the proper handling, use and care of a light microscope.
3. Be familiar with the markings on the individual objective lenses and their meanings.
4. Be familiar with the similarities and differences between animal and plant cells.

Materials and equipment

- Compound microscope
- Plain slides
- Cross section of a stem prepared slide
- Coverslips
- Ocular micrometer
- Toothpicks or cotton swabs
- Methylene blue stain
- Fresh white onions
- Lens cleaning solution

Background

The microscope is an indispensable tool in the study of cells. Anton van Leeuwenhoek (1632-1723) first observed protozoans using simple microscopes with a single lens to magnify the image. Today, compound microscopes have a two-lens system that achieves much greater magnification with greater resolution.

The objective of this lab is for you to become familiar with the use of compound microscope and to review its parts while learning slide preparation techniques, measuring techniques, and observing different types of cells.

For online laboratories, use the BioNetwork's Virtual Microscope (https://www.ncbionetwork.org/iet/microscope/).

1. Select the "Guide" box, then choose "Microscope care" from the menu.
2. Go over the 3 slides presented. Make sure you become familiar with how you must handle the microscope.

The compound microscope you will be using in this lab is shown in Figure 1. Before you begin working with the microscope, you should be familiar with all its parts.

For online laboratories, use the BioNetwork's Virtual Microscope (https://www.ncbionetwork.org/iet/microscope/).

1. Select on the "Guide" box, and then choose "Overview" from the menu.
3. Go over the 12 slides presented. Make sure you become familiar with how you must handle the microscope.

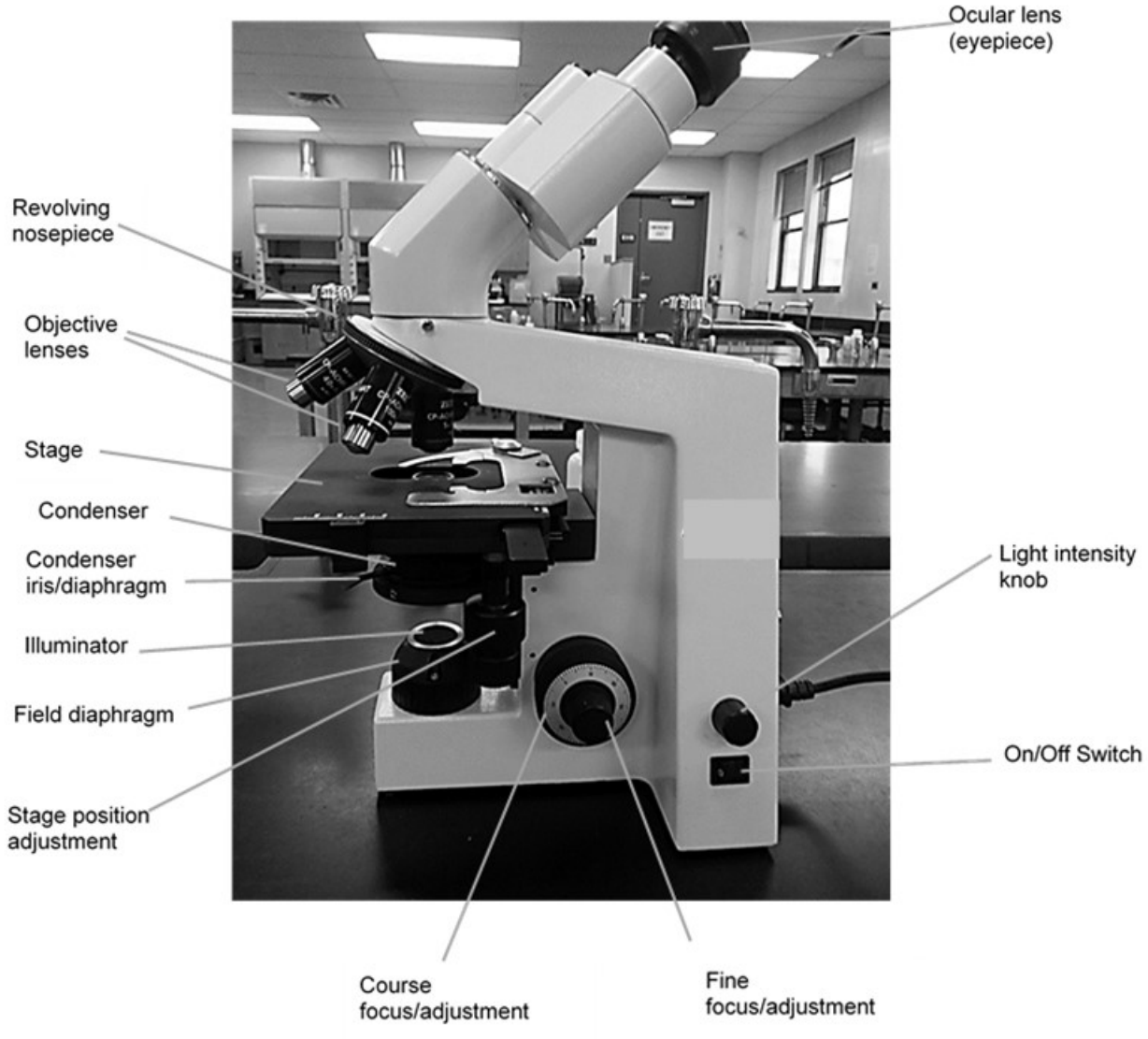
Once you have completed reviewing the 12 slides, continue with the activities below.

1. The **illuminator** is built into the base of the microscope and the light that is produced here comes from a high intensity bulb. The size of the illuminated field produced can be regulated by the **field diaphragm**. Each objective lens will require different sizes of illuminated fields to work optimally.

2. Light then passes to the **condenser**, which consists of an adjustable system of lenses that focus the light on the specimen. The **condenser/iris diaphragm** controls the diameter of the light beam entering the condenser. Both the **field diaphragm** and the **iris diaphragm** must be centered and adjusted in order to get the optimum illumination that will allow the best resolution for each objective lens and the ocular lenses. When light is not centered, there will be scattering.

3. The **objective lens** produces an enlarged and inverted projection of the object, while the **ocular lenses** produce the final image that is further enlarged and still inverted.

Figure 1. Binocular compound microscope with parts labeled



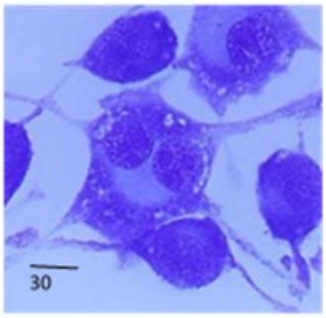
Magnification and resolving power

- Microscopes vary in magnification and resolving power.
- *Magnification* is the ratio of an object's image to its real size.
- *Resolving power* is a measure of image clarity.

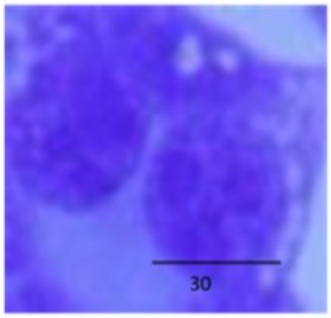
Figure 2. Difference between magnification and resolving power

Picture of puppy

Same picture of puppy magnified



Picture of Microglia



Picture of Microglia magnified

An image can be magnified but that does not increase the level of detail that can be observed, as can be seen in the pictures in Figure 2, where the magnified images appear fuzzy. The detail observed when looking under a microscope depends on the resolving power of a microscope

Questions

Before going on to the next section, answer the following questions.

1. In your own words, explain what is meant by the resolving power of a lens.

2. In your own words, explain what is meant by the magnifying power of a lens.

Procedures Part I

Follow the instructions below and during the experiment record your data in the tables and space given to you for this purpose. Write all the information during the lab.

The following are the different sections you will be completing during the first period of this lab session (approx. 1 hour):

1. Familiarization with the Microscope Components.
2. Measurement of field diameter for 10X

1. Familiarization with Microscope Components

- Familiarize yourself with your microscope by identifying the components labeled in Figure 1.
- Note the magnification of each objective and the ocular on your microscope and compute the total magnification with each objective and enter the values in Table 1.

- **For online laboratories**, use the BioNetwork's Virtual Microscope (https://www.ncbionetwork.org/iet/microscope/).

  a. Click on the "Guide" box, then choose "Objective lenses" from the menu.
  b. Go over the 4 slides presented. Make sure you become familiar with the lenses in a microscope and complete the table below.

  After completing the table, return to the main page in the link above and click on the "test" box. Choose the option "Calculating magnification" from the menu and complete the quiz.

  Take a screen shot of the page with your grade certifying completion and upload it in the box below.

Click/tap the box to upload your completion certificate.

Table 1. Magnification

Objectives	Ocular lens	Total magnification
10X low power		
40X high dry		
100X oil-immersion		

2. Measurements

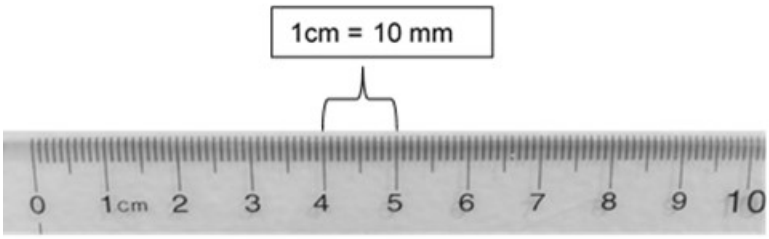
For online labs, use the pictures in *Appendix 1* to complete the exercises below. All pictures were taken while using the 10X objective. For the cheek cell exercise, see instructions under Part II C. Cheek epidermal cell: Observing the structure of animal cells.

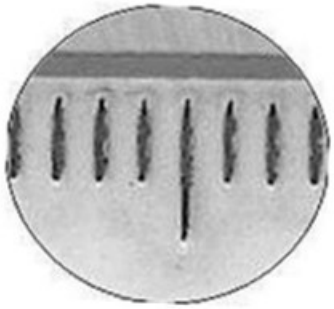
A. Determine the field diameter of your field of view: use metric system.

To estimate the size of the structures you will be viewing, you can measure the diameter of your field of view for the 10X lens using a transparent ruler.

You must use the metric system. You will be expected to know all the conversion factors in the metric system.

Figure 3. Transparent ruler with metric measurement units for length





Example of what the ruler will look like under the microscope.

Steps:

1. Place a transparent plastic ruler, like the one shown in Figure 3, on the stage and focus, using the 10X objective, on the lines on the ruler.

2. Once you have focused the ruler, count how many divisions can be seen from one edge to the opposite edge (the diameter of the field). Each small division represents 1 mm.
   How many mm is the field diameter when using the 10X objective? _____

3. Convert the total length of the field diameter from mm into µm. Remember that 1 mm = 1000 µm. _____

B. Determine the size of cells in a cross section of a stem.

Steps:

1. Examine a prepared slide from a cross section of a plant stem. Use your 10X objective.

2. Choose an area of the stem where you can clearly see the cell walls, and which will allow you to count how many cells fill one line across the diameter of your field of view. Choose an area where all cells are roughly the same size.

3. Count how many cells across the field of view.

4. Calculate the average diameter of each cell. To do this, remember that you determined the diameter of your entire field of view, so:

Average size of one cell = $\frac{\text{total diameter of the field of view } (\mu m)}{\text{total number of cells}}$

Average size of one cell = _____

Show all your computations below:

Procedures Part II

Follow the instructions below and during the experiment and draw what you are observing in the spaces provided. You must record all the information requested in this lab handout during the lab period.

Drawing and calculating your drawing magnification: For each one of these exercises please make sure you complete the following:

- Drawings of what you observe should be done **in pencil.**
- Each drawing should be properly labelled.
- You must record the size of each object you are observing to be able to calculate your drawing magnification.

1. Preparation of wet mounts, drawings and calculating magnification

In a wet mount, a piece of tissue is placed on a clean slide with a drop of water, stain or reagent. A coverslip is gently lowered on the preparation. In this lab section, you will be preparing two different wet mounts: onion epidermis and elodea.

A. Onion Epidermis: Observing the structure of plant cells

1. Add a small drop of water to the center of a clean slide.

2. Take a fresh piece of onion and remove a layer.

3. Using forceps strip a small piece of epidermis from the concave surface of a layer and place it on the drop of water, being careful that it does not fold over on itself. Add a drop of water and a coverslip.

4. Use the 5X (scanning) objective to find your specimen and focus using the coarse adjustment. Without touching the adjustment knobs, rotate the revolving nosepiece and place the 10X objective over your slide.

5. Examine the cells with the 10X and the 40X objectives; use the fine adjustment to focus.

6. Slowly close the condenser diaphragm while looking at your slide.

   A. What do you observe when you close the condenser diaphragm?

   B. How does the opening of the condenser diaphragm affect the contrast?

7. Add one or two drops of Haemalum acid solution at one edge of the coverslip and draw it through by touching a piece of paper towel to the opposite edge of the coverslip. **Wait 10 minutes for the onion skin to stain.**

8. Examine the stained cells with the 10X and 40X objectives, varying the opening of the **iris diaphragm** until the nucleus is clearly visible.

Table 2. Measurements of onion cells

Cell	Width	Length
1		
2		
3		
4		
Average		

B. Onion epidermal cell drawing

In the space provided below, draw one typical onion epidermal cell, and label the visible cell structures. Drawings should be done in pencil (for in person labs) or with drawing tools for online labs and should reflect what you see when looking at the structures under the microscope**.**

Every drawing done should have the following information:

- Name of organism (*Allium cepa* in this case)
- Cell type
- Stain or technique used for the preparation
- Drawing magnification (**size of your drawing of object) / (actual size of object)**

Draw and label one typical onion epidermal cell

Stain: _____

Actual Size: _____

Plate/Drawing Magnification: (size of your drawing of object) / (actual size of the cell) _____

Questions

After you finish your drawing, answer the questions below:

1. How does using a stain change the visibility of the structures? Why?

2. What features can you see under the microscope that allow you to characterize these cells as eukaryotic cells?

3. Plant cells are surrounded by a plasma membrane and they also have cell walls. What are the major components of plasma membranes? What is the major component of plant cell walls?

C. Cheek epidermal cell: Observing the structure of animal cells

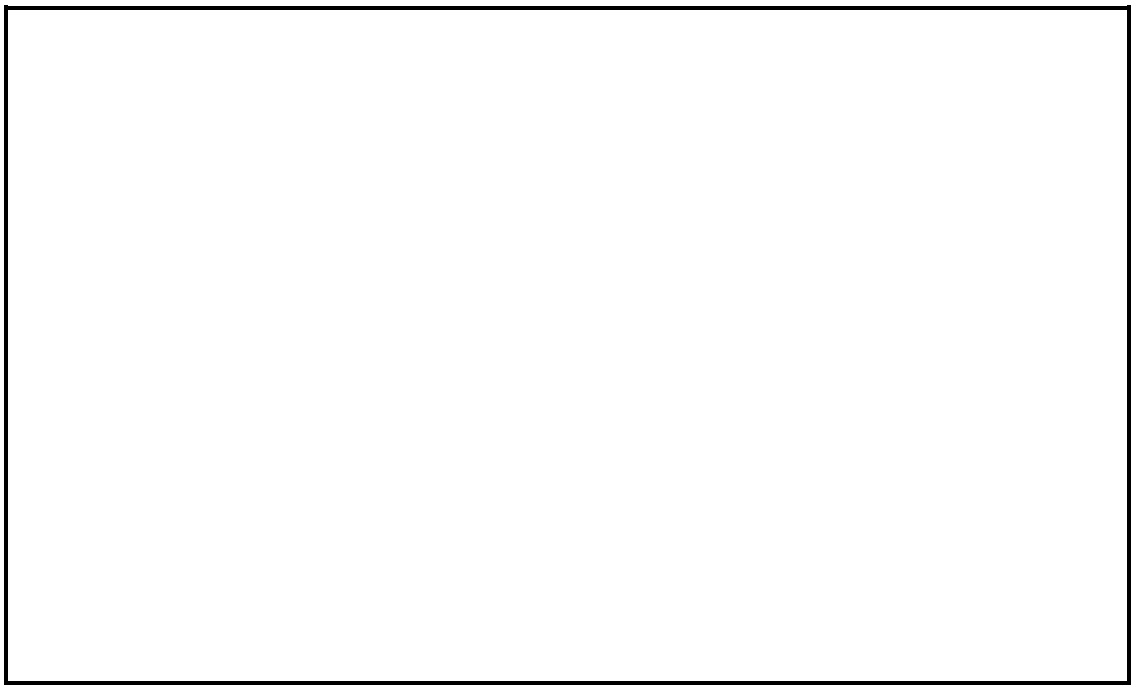
For online laboratories, you will be observing blood cells, not cheek cells. Use BioNetwork's Virtual Microscope (https://www.ncbionetwork.org/iet/microscope).

a. Select the "Explore" box, then choose "Slide catalog" from the menu.
b. Select the box that says "Human". From there, choose "Blood" and use the buttons for the coarse adjustment, the fine adjustment and light to focus on the cells.
c. For your drawing, use either the 40X or the oil immersion objective.

1. Put a drop of methylene blue stain on a slide.
2. Gently (lightly) scrape the inside of your cheek with the flat side of a toothpick.
3. Stir the end of the toothpick in the stain and throw the toothpick away.
4. Place a coverslip onto the slide; if you have excess stain, use a small piece of paper towel to draw the excess stain.
5. Use the 5X (scanning) objective to find the area with cells and focus. You probably will not see the cells at this power.
6. Switch to the low power (10X objective) by turning the revolving nosepiece. You should be able to see the cells although they will appear as small purplish little "clouds". Any large objects you see are probably crystals from the stain, not cells.
7. Once you think you have located a cell, switch to high power (40X) and refocus using ONLY the fine adjustment.

In the space below, draw one typical cheek epidermal cell, labeling the visible cell structures. **Drawings should be done in pencil (for in person labs) or with drawing tools for online labs and should reflect what you see when looking at the structures under the microscope.**

Draw one typical cheek epidermal cell or, alternatively, several blood cells



Stain: _____

Actual Size: _____

Plate/Drawing Magnification: (size of your drawing of object) / (actual size of the cell) _____

Question

What differences could you see, using a microscope, between the onion cells and the cheek cells (or alternatively, the blood cells)? Compare structures and size

First and last name: _____

Follow your instructor's directions in renaming and submitting your lab.

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Appendix

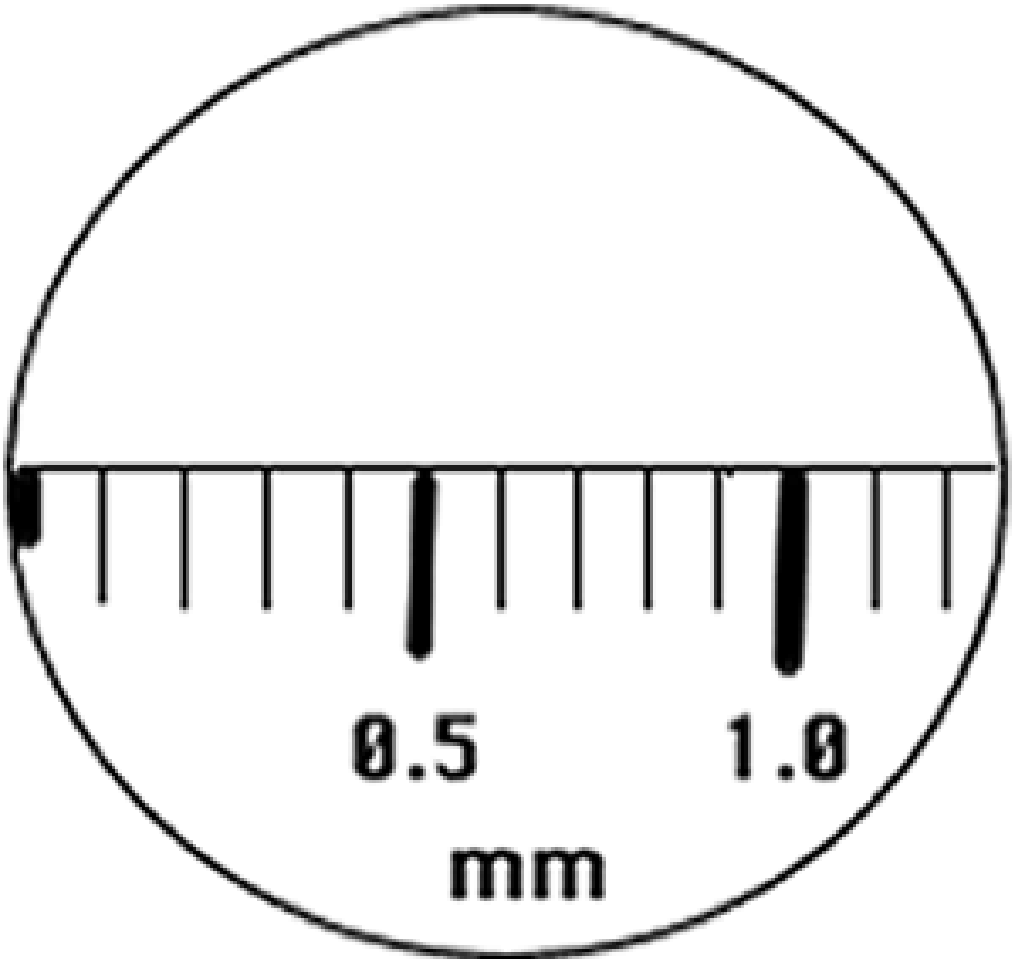
For online labs, use the pictures and diagrams to answer the questions requiring the figures.

Part 1. A Diagram

A. Determine the field diameter of your field of view: use metric system.

Use the following diagram to answer questions 2 and 3 from this section. This diagram represents the ruler seen when using your 10X objective lens. Note that this image is magnified.

Figure 4. A ruler viewed under a microscope



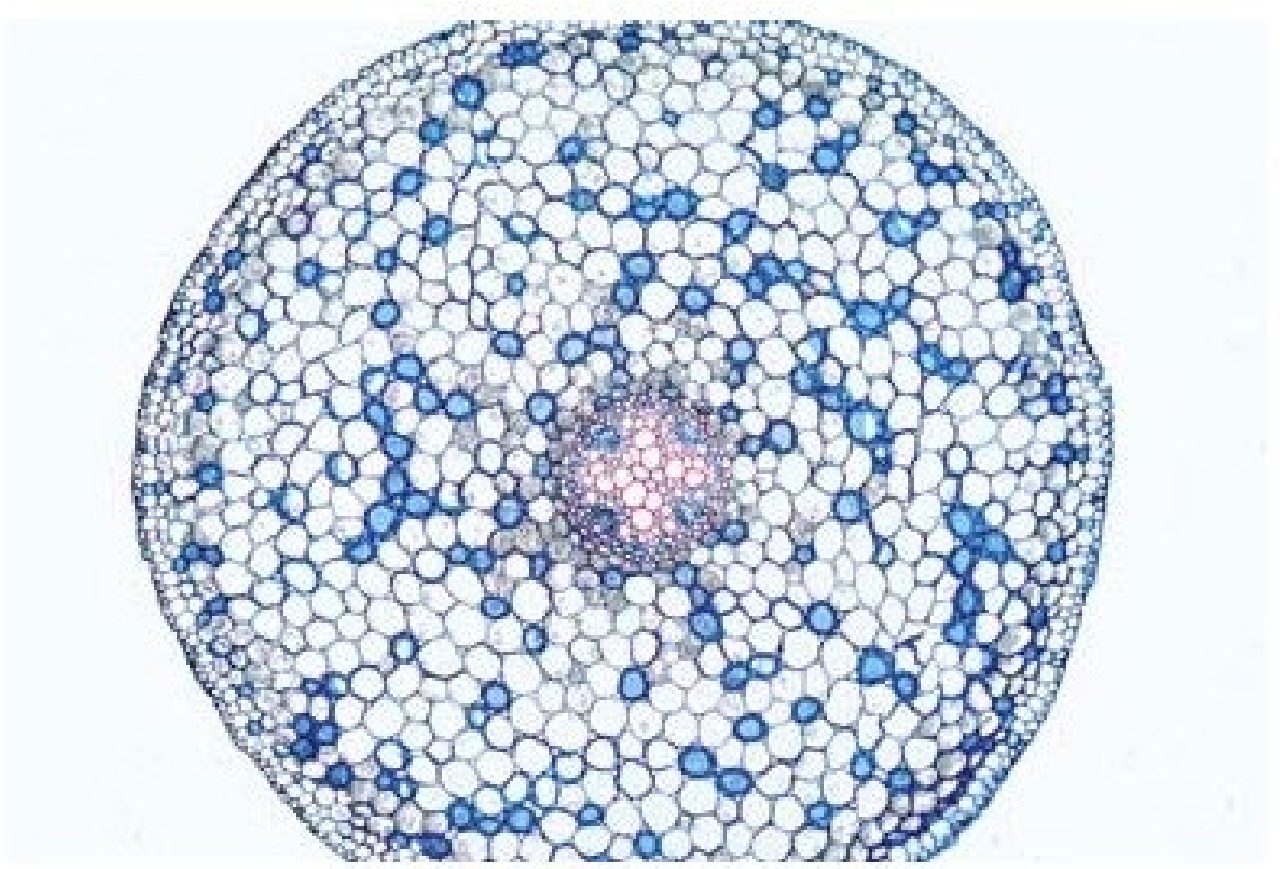
Part 1. B Picture

For **part B** of this question (record your answer to question 4 of part B), use the cross section of a stem of a plant (*Ranunculus sp.)* shown below.

To make it easier for you to calculate the size of the cells, you can divide the section in two, count and then multiply your result by 2 in order to determine the total number of cells.

Follow the instructions in your manual for your calculations.

Figure 5. Cross section of a stem of a plant



Part II. A Picture

For the questions on the onion epidermis, follow the instructions in your manual and use the picture below.

The following is a wet mount preparation of onion epidermis, taken under the 10X objective. Read the instructions in your lab manual and answer the questions.

Calculate the length and width of the cells and enter the values in the tables in your manual. Have each member of your group use a different row of cells to make their calculations.

Figure 6. Wet mount preparation of onion epidermis