Measurement – Lab Exercises

Exercise 1: Calibrate the microscope and measure a specimen

Measuring the size of specimens is an important technique to learn. You should have an approximate idea of the size of the organisms and structures we study in this lab. In this exercise, you will calibrate your microscope in order to measure specimen size. Although you calculated the total magnification in Part 1, microscopes have slight variations that require an instrument-specific calibration. You can use the eyepiece reticle to perform this calibration

The stage micrometer is a slide with a 1mm line on it. The line is divided into 100 even lengths. Each division is how large? (remember 1mm = 1000µm, µm = micrometer, or simply micron)

1 division = _____ mm
1 division = _____ µm

Materials

- Compound microscope with eyepiece reticle
- Stage micrometer – this is a slide with a ruler

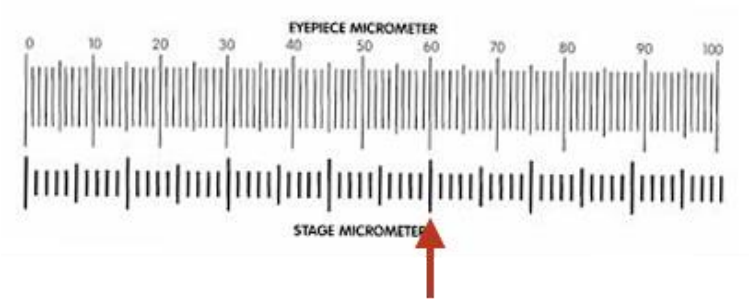
1. Turn the eyepiece until the eyepiece reticle is parallel with the stage micrometer.
2. Line up the 0 line of the eyepiece reticle with the 0 line of the stage micrometer.
3. Find a point where the lines on the eyepiece reticle and stage micrometer line up.
4. If you are confused by which ruler is the stage micrometer and which is the eyepiece reticle, you can close one eye, then the other. The stage micrometer will be visible to both eyes, but the eyepiece reticle will only be visible to one eye.
5. Calculate the relationship between the two:

Distance between two lines of the eyepiece reticle =
(number of stage micrometer divisions/number of eyepiece reticle divisions) x 10 µm

6. Repeat the procedure for each objective and fill in the table below

Objective	Number of stage micrometer divisions	Number of eyepiece reticle divisions	Distance between two lines of eyepiece reticle

If you are having trouble figuring out how to calibrate, see the figure and example below:



In this example, the eyepiece reticle is on top and the stage micrometer is on the bottom. They line up well in the place indicated by the red arrow. At this location, there are 60 eyepiece reticle divisions to 40 stage micrometer divisions. You would calculate the distance between two lines of the eyepiece reticle as follows:

Distance between two lines of the eyepiece reticle =
(40 stage micrometer divisions/60 eyepiece reticle divisions) x 10 µm = 6.67 µm/eyepiece reticle division

You would now be able to remove the stage micrometer and measure specimens using the eyepiece reticle. Remember that the calibration changes when you change objectives.

Now, obtain a prepared slide of a prokaryote and a eukaryote.

Using your calibration, measure the diameter of each cell (in µm) and record your results below:

Diameter of prokaryote = _____

Diameter of eukaryote = _____

Please take a picture of ONE of the specimens you image today. You may choose: the prokaryote or eukaryote you just measured, OR the red blood cell smear, OR one of the *Helianthus* slides. Make sure your picture is in focus, center and crop appropriately, and add a scale bar. Submit your image on Canvas.

Exercise 2: Measure a population of red blood cells (RBCs)

1. Obtain a slide with a blood smear. Using the calibration you calculated for your microscope last week, measure the diameter of 10 red blood cells.

2. Record your data in the table below. **Add your data to the class spreadsheet.**

1	2	3	4	5	6	7	8	9	10

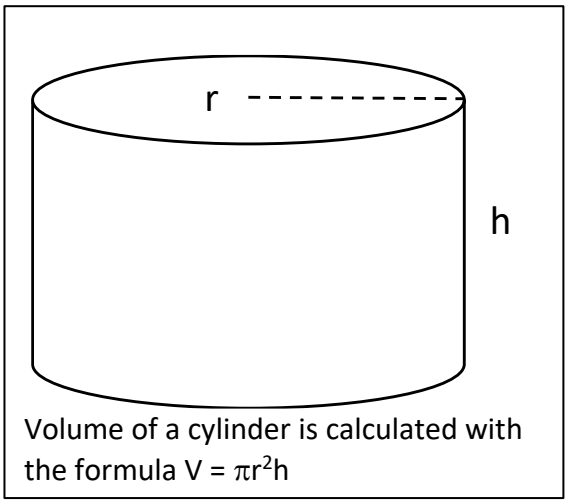
3. What is the mean diameter of the 10 RBCs? _____

4. Now calculate the **range** of this dataset. _____

5. Calculate the mean area of the visible surface of the RBCs. Use the formula for calculating the area of a circle. (r=radius, which is half the diameter)

   $A = \pi r^2$

   Mean area of RBCs: _____



Volume of a cylinder is calculated with the formula $V = \pi r^2 h$

6. Now, calculate (estimate) the mean volume of a red blood cell using the formula for the volume of a cylinder.[1] Assume that the height is 2.25μm. Include the unit. (Recall that 1 mL = 1 cm^3.)

   $V = \pi r^2 h$

   Mean volume of red blood cells: _____

7. Calculate the mean surface area of the red blood cells, using the formula for calculating surface area of a cylinder.

   $SA = 2\pi rh + 2\pi r^2$

   Mean surface area of red blood cells: _____

8. Imagine the height of the cylinder is doubled.
   a. Will surface area or volume increase more?

   b. Will the surface area to volume ratio (SA/V) increase or decrease?

9. How variable is this population of cells? How would you explain the observed variation in RBC size, given their function?

[1] Red blood cells are not cylinders, but rather biconcave disks. However, using the formula for a cylinder gives a very close approximation of the volume (Ion Udroui, Estimation of erythrocyte surface area in mammals 2014).

Exercise 3. Measure Plant Parenchyma Cells

Introduction: For this exercise, you will consider the same population of plant cells, sectioned in two different planes.

Most plants are formed of three types of tissues:

1. **Vascular Tissues** that rapidly conduct fluids within the plant body. There are two types of vascular tissues, **xylem** (examined in Exercise 4) that functions in the rapid conduction of water, and **phloem** that rapidly conducts a fluid (phloem sap) that contains sugars and other small organic molecules.
2. **Dermal Tissues** that surround and protect the plant body. These include the **epidermis** and **cork**. Epidermis usually consists of single layer of cells that surrounds leaves, flowers, and non-woody stems and roots. Woody stems and roots are surrounded by a thick layer of cork (familiar as the outer layer of "bark").
3. **Ground Tissues** that carry out most metabolic operations of the plant. Ground tissues may be photosynthetic or specialized for storage or other purposes. The most common cell type in ground tissues is **parenchyma** (from the Greek for "poured in"). Parenchyma is the focus of this lab exercise.
    a. Parenchyma cells have thin, stretchable, primary cell walls, and are usually living at functional maturity (note: this last statement may seem unnecessary, but some plant cell types such as the conducting cells of xylem do not begin functioning until they die). Parenchyma cells are highly variable in size, function, and shape, and in some parts of the plant (such as the stem) they act as a "filler" that enlarges to fill available space. Although highly variable in shape, unspecialized parenchyma cells are generally considered to approximate an elongated dodecahedral (20-sided polygon) shape when packed together – this is the same shape that a sphere would take if compressed with other spheres (think copper bbs or soap bubbles packed together such that their abutting surfaces are flattened).

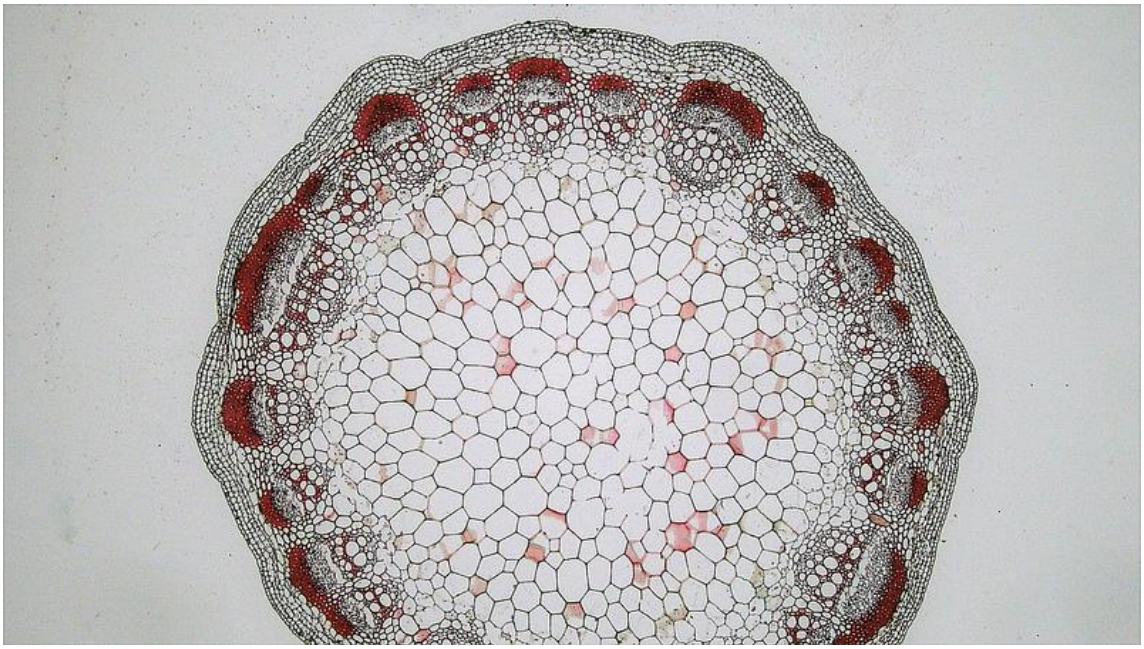
In this lab exercise you will be observing and measuring variation in parenchyma size in *Helianthus* (sunflower) stems.

Materials:

- ***Helianthus* combination slide (showing cross sections and longitudinal sections).**

Procedure:

1. Observe a prepared slide of a *Helianthus* stem in cross section (see image below):



Orientation (What am I looking at?):

- Outer layer of cells: epidermal cells.
- Three to five cell layers just within the epidermis: collenchyma cells.
- Vascular bundles are the most striking features of the stem. In cross section they appear oval to round. These are made up of vascular tissues.
- All of the cells with thin cell walls that "fill in" between the vascular bundles and collenchyma are parenchyma cells. These are the cells of interest for this lab exercise.

*The safranin-fast green staining technique is a differential staining technique commonly used on plant tissues. Plant cells are initially stained with the red stain Safranin. This is a "slow" stain that penetrates cell structures (especially cell walls) slowly (over a period of hours). The slide is briefly "de-stained," clearing safranin from thin cell walls and structures that bind the stain weakly. Following de-staining, the slides are counter-stained with fast green (for 15 to 30 seconds) to re-stain the portions of the cell cleared in by de-staining. Nuclei, some organelles, and thickened (secondary) cell walls usually retain the red stain.

a. Measure the diameter of 10 parenchyma cells located in the outer portion of the stem, just inside of the collenchyma layer. Record your measurements in the table below.

b. Measure the diameter of 10 parenchyma cells from the central portion of the stem. Record your measurements in the table below. **Add your data to the class spreadsheet.**

Size of outer stem Parenchyma cells:

1	2	3	4	5	6	7	8	9	10

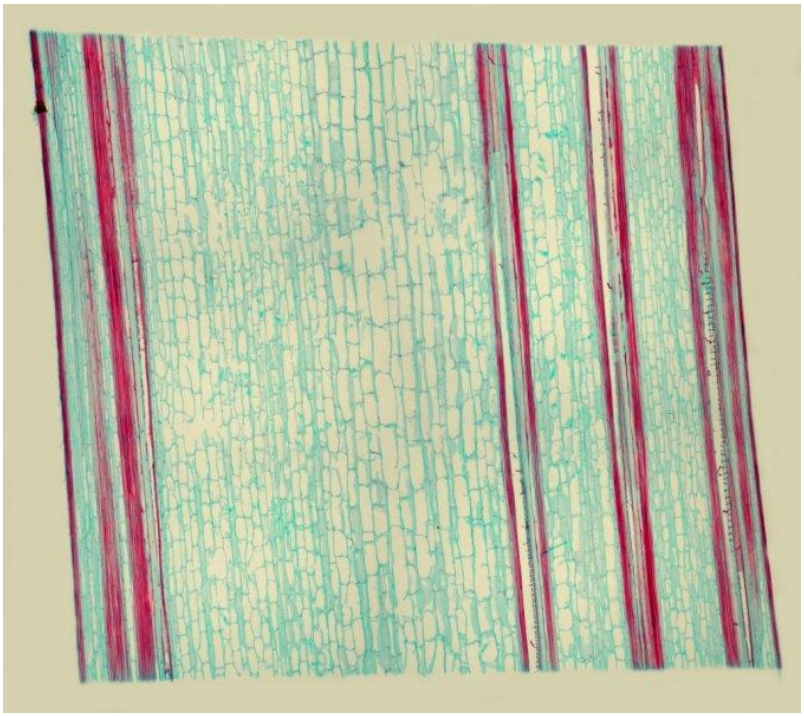
Mean diameter (micrometers): _____

Size of central stem Parenchyma cells

1	2	3	4	5	6	7	8	9	10

Mean diameter (micrometers): _____

2. Observe a prepared slide of a *Helianthus* stem in longitudinal view (see image below):



As was seen in the cross section, the parenchyma cells will have thin cell walls and stain green.

a. Measure the height of 10 parenchyma cells located in the outer portion of the stem, just inside of the collenchyma layer. Record your measurements in the table below.

b. Measure the height of 10 parenchyma cells from the central portion of the stem. Record your measurements in the table below. **Add your data to the class spreadsheet.**

Height of outer stem Parenchyma cells:

1	2	3	4	5	6	7	8	9	10

Mean height (micrometers): _____

Height of Parenchyma cells from the central stem region:

1	2	3	4	5	6	7	8	9	10

Mean height (micrometers): _____

Use your measurements from above to calculate the average volume of parenchyma cells from the outer versus central regions of the stem. Although considered to be polyhedral, parenchyma cells are roughly cylindrical and their volume may be approximated using the formula: $\pi r^2 h$.

Average volume of parenchyma cells from the outer stem region: _____

Average volume of parenchyma cells from the central stem region: _____

What is the range of observed parenchyma cell diameter? _____

What is the range of observed parenchyma cell height? _____

1. How does your analysis of parenchyma cell size, shape, and volume compare with your similar analysis of human red blood cells? Which cell type is more variable?

2. Thinking of the formation and function of these two cell types (parenchyma versus red blood cells), why do you think these two cell types are so different in terms of their size variances?