BIOLOGY Paper 3

Manipulation, measurement and observation

Decisions relating to measurements and observations

For investigations:

- Identify the independent variable and dependent variable
- Decide a suitable range of values to use for the independent variable at which measurements of the dependent variable are recorded
- Decide the number of different values of the independent variable (a minimum of five) and the intervals between them
- Decide how to change the value of the independent variable
- Decide how the dependent variable should be measured
- Decide the number of replicates at each value
- Decide on appropriate controls for the experiment or investigation
- Decide which variables need to be standardised and how to standardise them. (Variables expected to have a minimal effect, such as variation between test-tubes of the same type, do not need to be standardised.)

For microscopy:

- Set up a light microscope to view and observe specimens
- Follow instructions to find and draw particular tissues in plant and animal specimens and label the drawings appropriately
- Follow instructions to find and draw particular cells and structures within the cells
- Make a temporary slide of stained cells or tissues
- Calculate actual sizes of tissues or cells from measurements of photomicrographs, using magnifications, scale bars or representations of eyepiece graticules and stage micrometres
- Estimate the number of cells or cell organelles in a given area using a sampling method, such as grids or fields of view

Collection of data and observations

For investigations:

- Follow instructions to collect results
- Consider the hazards of the procedure, including the use of any solutions and reagents, and assess the risk as low, medium or high
- Take readings to obtain accurate data (quantitative results) or observations (qualitative results).

For microscopy:

- Draw plan diagrams to show the distribution of tissues in a specimen, with no cells drawn and the correct proportions of layers of tissues

- Draw the observable features of cells in a specimen showing:
    - the correct shapes
    - the thicknesses of cell walls where applicable (drawn with two lines or drawn with three lines where two cells touch)
    - the relative sizes and proportions – observable cell contents only
- Measure tissue layers or cells from photomicrographs using a ruler or given scale, including representations of eyepiece graticules
- Make accurate observations from specimens including counting numbers of cells or cell organelles
- Record similarities and differences between two specimens using only their observable features.

Presentation of data and observations

<u>Recording data and observations</u>

For investigations:

- Record raw results (unprocessed) and calculated results (processed) in an appropriate table with:
    - descriptive headings, including required units (no units in body of table)
    - heading for the independent variable to the left of (or above, if the table is in rows) the dependent variable
- Record quantitative data to the number of decimal places that is appropriate for the measuring instrument used
- Record qualitative observations using clear descriptions
- Record calculated values (processed results) in an appropriate table.

For microscopy:

- Record the fine details of the specimen, including drawing the detailed shapes of layers or outlines of specimens in plan diagrams and drawing the shape and position of observable cell organelles in cells.

<u>Display of calculation and reasoning</u>

For microscopy:

- Display calculations clearly, showing all the steps and reasoning
- Use the correct number of significant figures for calculated quantities. This should be the same as, or one more than, the smallest number of significant figures in the data used in the calculation.

<u>Layout of data and observations</u>

- Display data as a graph (continuous data), bar chart (discontinuous or categorical) or histogram (frequency data)
- Draw a graph, bar chart or histogram clearly and accurately with:
    - independent variable on the x-axis and dependent variable on the y-axis

    - axes labelled to match the relevant table headings, including units where appropriate
    - a scale where both axes should use most or all of the grid available and allow the graph to be read easily to within half a square
    - all graph points plotted accurately using a sharp pencil, as a small cross or a small dot in a circle, with the intersection of the cross or centre of the dot exactly on the required point
- The plotted points of a graph connected with a clear, sharp and unbroken line, either as a line of best fit, a smooth curve or with ruled straight lines joining the points
- No extrapolation of graph lines unless this can be assumed from the data
- All bars on a bar chart or histogram plotted accurately, with clear, unbroken lines that are drawn with a sharp pencil and ruler.

For microscopy:
- Make drawings, using a sharp pencil to give finely drawn lines that are clear and unbroken
- Make drawings that use most of the available space and show all the features observed in the specimen, with no shading
- Organise comparative observations, showing differences and similarities between specimens

Analysis, conclusions and evaluation

<u>Interpreting data and observations</u>

For investigations
- Calculate an answer with the correct number of significant figures using quantitative results or data provided
- Use a graph to find unknown values
- Estimate the concentrations of unknown solutions from qualitative results
- Identify the contents of unknown solutions using biological molecule tests
- Identify anomalous results and suggest how to deal with anomalies
- Describe patterns and trends using the data provided in tables and graphs
- Evaluate the confidence with which conclusions might be made.

For microscopy:
- Calculate an answer with the correct number of significant figures using quantitative results or data provided
- Compare observable features of specimens of biological material including similarities and differences between specimens on a microscope slide and specimens in photomicrograph

Drawing conclusions

From results, observations or information provided:

- summarise the main conclusions
- state and explain whether a hypothesis is supported
- make predictions from the patterns and trends in data
- suggest explanations for observations, results, patterns, trends and conclusions

Identifying sources of error and suggesting improvements

- Identify systematic or random errors in an investigation, understanding that systematic errors may not affect the trend in results whereas a random error may affect the trend.
- Identify the main sources of error in a particular investigation
- Suggest improvements to a procedure that will increase the accuracy of the observations or measurements, including:
    - using a more effective method to standardise relevant variables
    - using a more accurate method of measuring the dependent variable
    - using smaller intervals for the values of the independent variable
    - collecting replicate measurements so that a mean can be calculated
- Suggest how to extend the investigation to answer a new question, for example by investigating a different independent variable or applying the method to a new context
- Describe clearly, in words or diagrams, improvements to the procedure or modifications to extend the investigation

Graphs

Line graphs

- x-axis = independent variable
- y-axis = dependent variable
- Choose a scale that will enable you to use all/most of the grid provided
- 1/2/5 rule: each 2mm square should be 1/2/5 units
- Use cross / encircled dot to plot points
- Best fit line/ smooth curve/ point-to-point joining is acceptable
- NO extrapolation, unless told to do so

Bar chart

- If data is discrete/ in categories, bars should be separate.
- If data is continuous, bars should be joined
- Don't shade bars

Division of marks

1. Axes: correct axes for independent & dependent variable, labelling + units
2. Scale: 1/2/5 rule, covers all/ most of the grid

3. Plots: small & accurate
4. Line: line passing through all points, smooth, unbroken, no extrapolation

Tables

- Make sure the table is fully lined
- Follow the instructions
    - Follow the order of independent variable: from highest to lowest/ lowest to highest
    - Results for all replicates must be recorded in the table (and mean, if asked)
    - When specified, record only the processed data; eg. measure the temperature every 30 seconds for 4 minutes and record the highest temperature: do not record all the temperatures, only the highest temperature!
- Headings
    - Independent variable should be in the top row/ leftmost column
    - Write the name of the variable in full
    - Units must be given in the heading, NOT in the body of the table
- Significant figures
    - Record quantitative data to the same number of sf (depends on measuring instrument)

Dilution methods

Simple dilution

Stock solution is used to prepare all the other concentrations.

Dilution formula

$M_1V_1 = M_2V_2$

M = concentration

V = volume

Eg. you have 4% glucose stock solution, and need to prepare 1% of 10 cm3 solution.

$M_1V_1 = M_2V_2$

$4V_1$ = 1 x 10

V_1 = 10/4 = 2.5cm3

Thus, 2.5cm3 of 4% glucose is needed to prepare 10cm3 of 1% glucose:
2.5cm3 of 4% glucose + 7.5cm3 of distilled water = 10cm3 of 1% glucose

Serial dilution

Stock solution is used to prepare 1st concentration, 1st concentration is used to prepare the 2nd, so on.
Used when you want to halve the concentration/ make it 10x more diluted

When concentration has to be halved:

Eg. you have 4% glucose stock solution, and need to prepare 10cm3 of 2% and 10cm3 of 1% glucose solution.

- Take 5cm3 of 4% glucose solution
- Add equal amount of distilled water (5cm3)
- This gives 10cm3 of 2% glucose solution
- Take 5cm3 of 2% glucose solution
- Add equal amount of distilled water (5cm3)
- This gives 10cm3 of 1% glucose solution

10x dilution:

Eg. Eg. you have 4% glucose stock solution, and need to prepare 10cm3 of 0.4% and 10cm3 of 0.04% glucose solution.

- Mix the solution with in water in 1:9 ratio
- Take 1cm3 of 4% glucose solution
- Add 9cm3 of distilled water
- This gives 10cm3 of 0.4% glucose solution
- Take 1cm3 of 0.4% glucose solution
- Add 9cm3 of distilled water
- This gives 10cm3 of 0.04% glucose solution

Eyepiece graticule, Magnification, Resolution

Eyepiece graticule

- Scale fitted inside the microscope's eyepiece.
- You can see the full scale as it is fitted near the eyepiece.

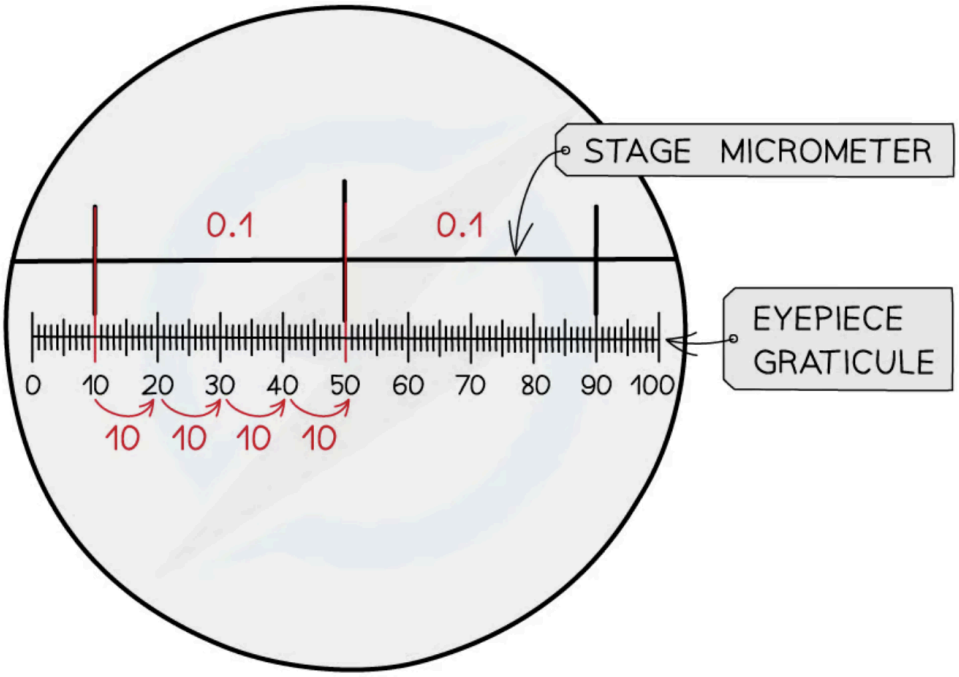
Stage micrometre

- Scale with a precise measurement etched onto its surface, placed on the stage of the microscope.
- You can only see part of it since it is magnified.
- It typically has markings such as 0.01mm apart.
- It is used to calibrate the eyepiece graticule.
- It allows accurate measurements of specimens under the microscope.

Steps to calibrate eyepiece graticule

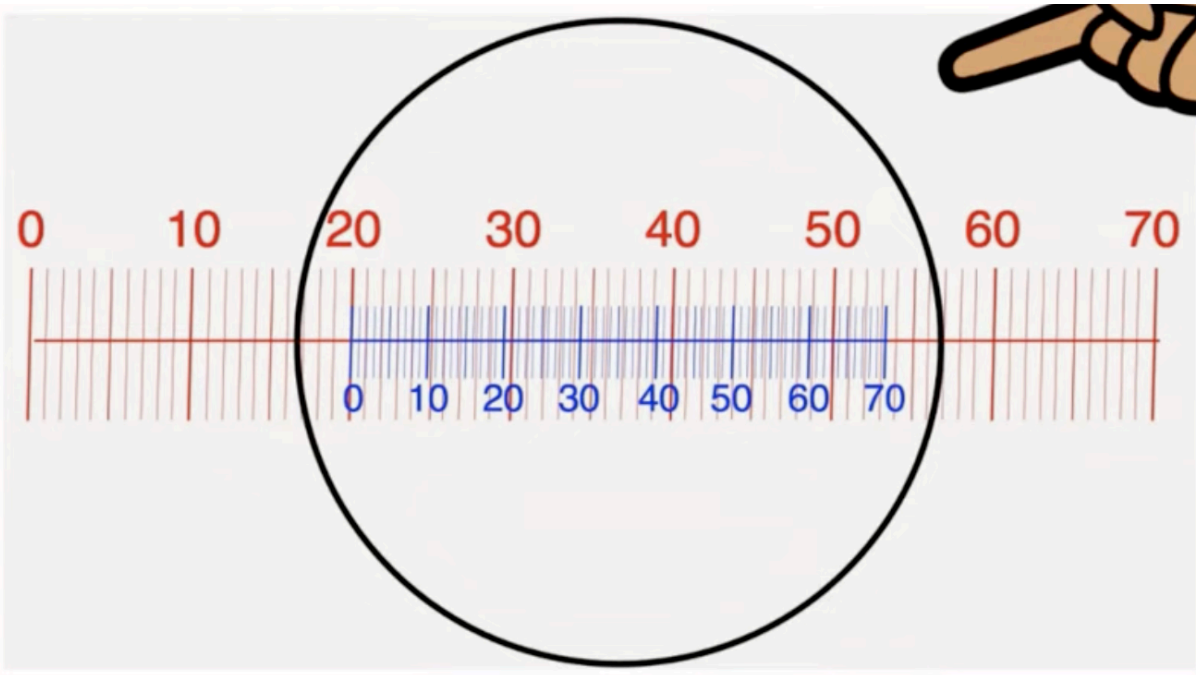
1. Align eyepiece graticule with stage micrometre, so they are on top of each other, horizontally.
2. Count how many divisions on eyepiece graticule corresponds to a set number of stage micrometre divisions.
3. Calculate how big one eyepiece graticule division is.
    - Check the size of the stage micrometer (in the label) to determine the length of every small division on the stage micrometer.

- If stage micrometer is 1mm long in total, every 10 small divisions is 0.1mm & every small division is 0.01mm

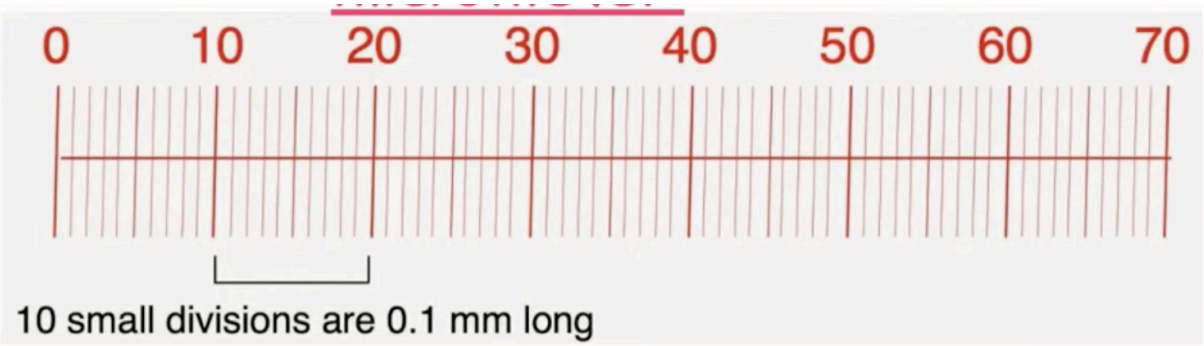


- 2 stage micrometre divisions of 0.1mm are visible; 0.1mm = 100 μm
- 40 graticule divisions = 100 μm
- 1 graticule division = number of μm ÷ number of graticule divisions
- 1 graticule division = 100 ÷ 40 = 2.5 μm = magnification factor
- length of object = graticule divisions covered by object x magnification factor

Example:



- Stage = red
- Eyepiece = blue
- Every 10 stage micrometre divisions = 21 eyepiece graticule divisions



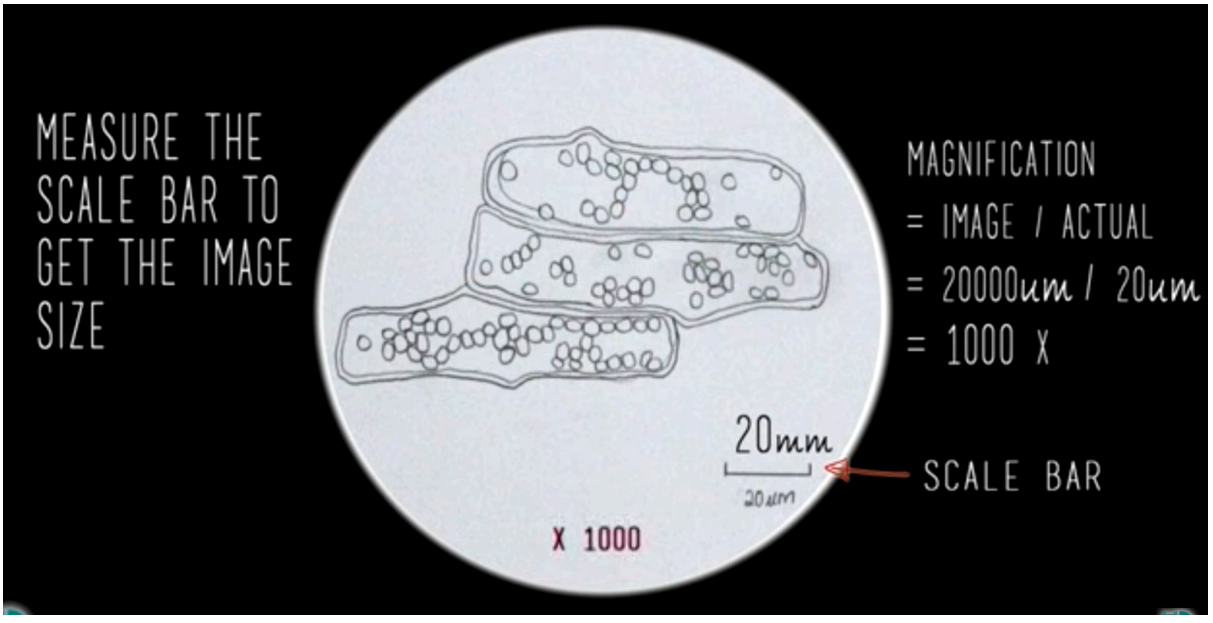
- 10 micrometre divisions = 21 graticule divisions
- 0.1mm = 21 divisions
- 0.1/21 = 1 division
- 1 division = 0.0048mm = 4.8μm

<u>After calibration</u>
Measure the specimen using eyepiece graticule, and convert the number of divisions on graticule to an actual length.

Magnification

Magnification = image size / actual size

Sometimes, a scale bar may be given in the question. Measure the length of the scale bar to get the image size.



Resolution

Ability of a microscope to distinguish 2 objects as separate from one another.
High resolution = able to distinguish objects which are very small and close to each other.
Small number for resolution = higher resolution = clearer image

- Maximum resolution of light microscope = 200nm
- Maximum resolution of electron microscope = 0.5nm

Biochemical tests

1. Quantitative
    - Shows the amount of a substance in a sample
    - Semi-quantitative: shows a rough estimate of the amount of substance

2. Qualitative
    - Shows the presence/ absence of a substance in a sample

Benedict's test

- Test for reducing sugars (monosaccharides + some disaccharides, eg. maltose)
- Test reagent = benedict's solution = blue

<u>Procedure</u>

- Add equal amounts of sample and benedict's solution (eg. 1cm3 of each) in test tube
- Place the test tube in water bath
- Temperature of water bath should be just above 80˚C (just boil it)
- Heat for 2-5 minutes

<u>Results</u>

- Positive result: green → yellow → brick red ppt
- Negative result: solution remains blue

<u>Explanation</u>

- Benedict's solution contains $CuSO_4$ (blue)
- Reducing sugars reduce the soluble $CuSO_4$ to insoluble red-brown ppt of CuO

<u>To test for presence of non-reducing sugars (like sucrose)</u>

- Add 2cm3 of sucrose solution in test tube
- To this, add 1cm3 of dilute HCl
- Heat in boiling water bath for 1 min
- Neutralise the mixture with $NaHCO_3$; keep adding until no effervescence
- Take 1-2cm3 of resulting solution and carry out Benedict's test

- Blue colour turns brick red

- When sucrose is heated with acid, it gets hydrolysed to glucose + fructose
- These are monosaccharides that give positive result with Benedict's solution
- Neutralisation with NaHCO3 is required to prevent false positive result due to presence of HCl

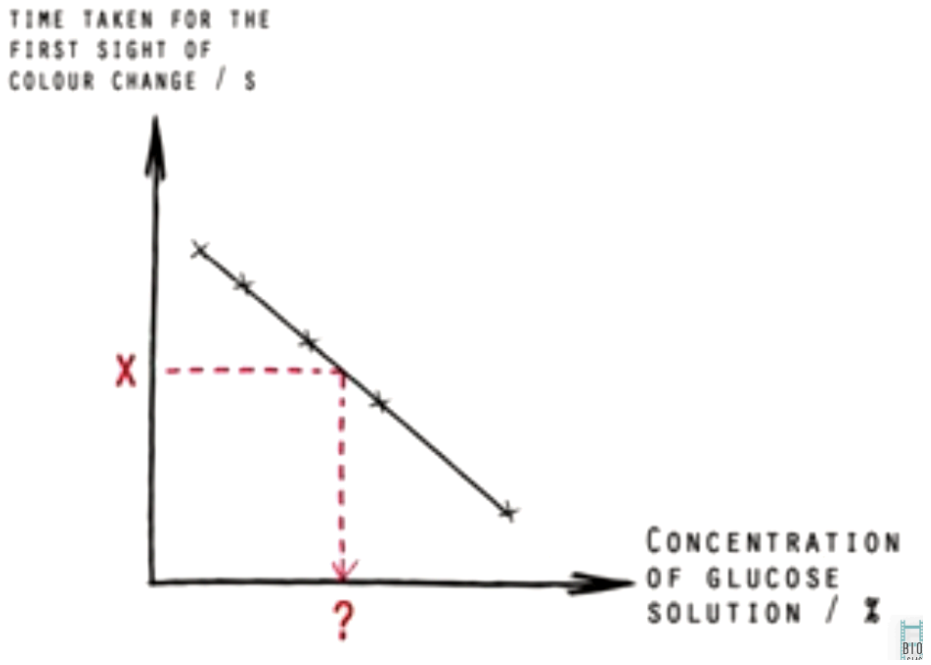
<u>Semi-quantitative benedict's test</u>

- Prepare a set of standard solutions: from a given stock solution, use serial dilution to prepare further solutions.
- Have at least 5 standard solutions in total.
- Carry out Benedict's test for all 5 solutions.
- Record results:
    1. Record time taken for the first sight of colour change OR
    2. Record final colour change after fixed time

If measuring time for first colour change:

- Start stopwatch once sample + Benedict's solution is placed in water bath
- Stop timer when colour starts to change & record
- Higher concentration = less time
- Plot results on a graph
- Carry out the same test for solution of unknown glucose concentration
- Use interpolation method to find unknown concentration

Interpolation



If recording colour change after fixed time:

- Carry out Benedict's test for all standard solutions for fixed time (eg. 2 min)
- This gives a series of colour standards
- Higher concentration = more intense brick red coloration

- Carry out the same test for solution of unknown glucose concentration
- Match its colour to the colour standard and estimate its concentration

Iodine test

- Test for starch
- Add few drops of iodine in potassium iodide solution to the sample
- Positive result: turns from brown to blue-black

Emulsion test

- Test for lipids
- Add equal amounts of sample and ethanol to test tube (eg. 1cm3 of each)
- Shake the mixture vigorously to dissolve the lipids
- Add equal amount of cold water (2cm3)
- Positive result: cloudy white suspension
- Lipids are soluble in ethanol but insoluble in water

Biuret test

- Test for proteins
- Add equal amount of sample and KOH solution in test tube and mix well
- Add few drops of $CuSO_4$ solution (blue)
- Positive result: turns from blue to purple

<u>Explanation</u>

- This is a test for peptide bonds
- Nitrogen atom in the peptide chain forms a purple complex with Cu^{2+} ions

Common Errors in Experiments

- Systematic errors occur throughout the experiments, as they result from uncertainties in measurements.
- Random errors differ across experiments as they arise due difficulties in controlling standardised variables and measurements of dependent variables.

- Anomalous readings are too low/too high/does not fit the trend and are usually discarded, and the experiment repeated.

- Difficult to judge/compare color/colour change using color charts.
- In experiments that use water-baths, temperature of the test tube falls when it is removed from the water bath.
- Fast changes in color/formation of bubbles can cause errors in timing, i.e., stopwatch started/stopped late/early.
- Difficult to start/stop stopwatch and add/remove samples at the same time.
- Difficult to judge and count the number of cells that are plasmolysed.
- In experiments where length/width/diameter of specimen is measured, error occurs due to difficulty in focusing both ruler and specimen at the same time; parallax error; thickness of ruler lines.
- In immobilizing enzyme reactions, error can occur due to sodium alginate beads:
    - sticking to the sides of the tube
    - sticking together with those already dropped
    - floating up under each other
- In experiments that require shaking of test tubes, errors can occur due to uneven shaking/varying forces used to shake the tubes.
- Errors can occur due to different sizes of bubbles, difficulty in counting the number of bubbles (fast formation).
- In experiments that require formation of drops using syringes, error due to different sizes of drops as pressure is applied on the syringe.
- In experiments that require measurements to be made using eyepiece graticule and stage micrometer, errors can occur due to
    - Difficulty in judging the edge of the specimen
    - irregular shape of specimen.
- Errors can occur due to decrease in Hydrogen peroxide concentration if left open, as H_2O_2 degenerates.
- Errors can occur if test tubes used are not dry, as droplets on water on sides of test tube can reduce experiment's reliability.
- Systematic errors occur throughout the experiments, as they result from uncertainties in measurements.
- Random errors differ across experiments as they arise due difficulties in controlling standardized variables and measurements of dependent variable.
- Anomalous readings are too low/too high/does not fit the trend and are usually discarded, and the experiment repeated.

Modifications & Improvements of experiments

- Use larger numbers of the prime sample of investigation (e.g. potato strips/leaves)

- Use digital balance to measure mass accurately.
- To keep surface area constant, use Vernier calipers to measure lengths and sharp scalpel to cut (e.g. potato strips).
- Use graduated pipettes to measure small volumes of solution accurately.
- In Benedict's tests, heat samples for the same amount of time.
- Carry out experiments for the same amount of time if time is not the independent variable. To do this, stagger start or carry out experiments separately.
- Carry out experiments at wider ranges of temperature/concentrations (at least 5)
- Make wider ranges of concentration using serial or simple dilutions.
- Repeat experiments at each temperature/concentration 3-5 times AND take the mean of results.
- Keep volumes of solution same throughout all the repeats (using graduated pipette/burette).
- During Benedict's tests, keep the temperature at around 80-100 degrees Celsius.
- Use the same volume of Benedict's solution in all tests.
- Cover H_2O_2 with lid/aluminum foil to prevent degeneration.
- Dry test tube with white towel or use new test tubes before starting each experiment
- To prevent evaporation, use rubber bungs in test tubes or cover the containers (air-tight).
- To standardize the position of delivery of solution into a test tube, make a mark on the test tube.
- Use a magnetic stirrer to prevent errors caused by uneven shaking of test tubes.
    - increase evaporation rates in experiments
    - Increase temperatures using thermostatically controlled water bath.
    - Increase wind speed using a fan (keep fan speed constant).
    - Decrease humidity using fan or
        - (calcium/sodium/potassium) hydroxide/chloride/oxide
        - Silica gel/drying agent
        - dehumidifier
- In experiments where oxygen is released, gas syringe can be used to collect oxygen; an oxygen sensor can be used to detect oxygen produced.
- Burettes can be used to make drop sizes similar since pressure is not applied as in syringes.
- To control the effects of light intensity, the lamp can be set at a constant distant/power.
- Place a container of water in front of the lamp to act as a heat shield.
- Alter light intensity by increasing power or adding more identical lamps.

- Other standardized variables include mass, concentration, volume, source, age, storage, conditions, and genotype of sample depending on the requirements of the experiment.

Formulas for calculations

- Take at least 5 measurements to increase accuracy of results.
- % error = $\frac{\text{No. of readings} \times \text{Half of smallest scale division}}{\text{Total reading}} \times 100\%$
- Mean = $\frac{\text{Sum of data}}{\text{No. of data}}$
  - Useful for replicated readings.
- Gradient = $\frac{\Delta y}{\Delta x}$, where Δy & Δx are height and width of triangle.
- Draw right-angled triangle from 2 points on straight line graph or tangent of curve; Ensure that triangle exceeds half of graph.
- % change = $\frac{\text{Final} - \text{Initial}}{\text{Initial}} \times 100\%$
- It makes comparing easier by negating effects of differences in initial readings between samples.
- Error in a measurement is half of the value of the smallest division on the scale you are reading from.
- If you read from two positions in the scale, i.e., initial, and final reading, the error is multiplied by 2 because error in measurements can occur twice
- To calculate percentage uncertainty, use the equation:

$$\text{percentage of uncertainty} = \frac{\text{uncertainty}}{\text{actual measurement}} \times 100$$

Quality of measurements

Term	Explanation	Improvement
Accuracy	Closeness to true value	Better instruments
Precision	Closeness to repeated readings	Control all variables
Reliability	Confidence in results	Repeat readings and take mean
Validity	Agreement between hypothesis and investigation	Check relation between key and derived variables

Drawings

<u>Plan diagrams</u>

Shows the distribution of tissues in a specimen

- Use lines to show the different types of tissue
- Show the relative thickness

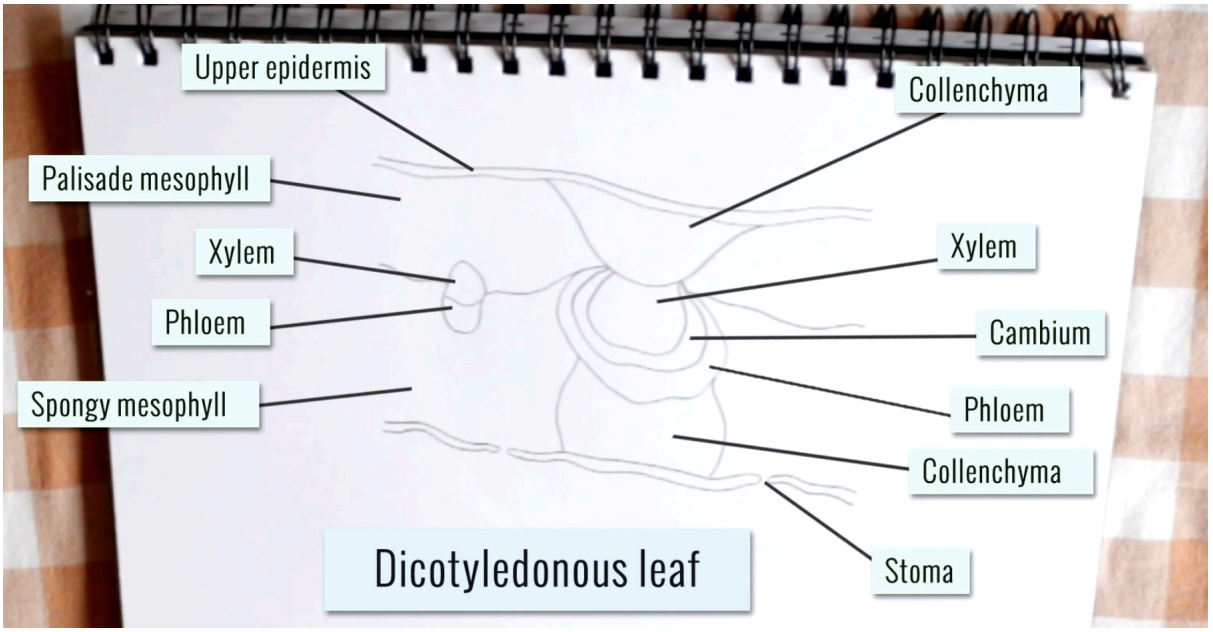
1. Quality of drawing
    - Clear, sharp, fine, unbroken lines
    - Not ruled
    - Do not shade
    - Don’t draw any cells

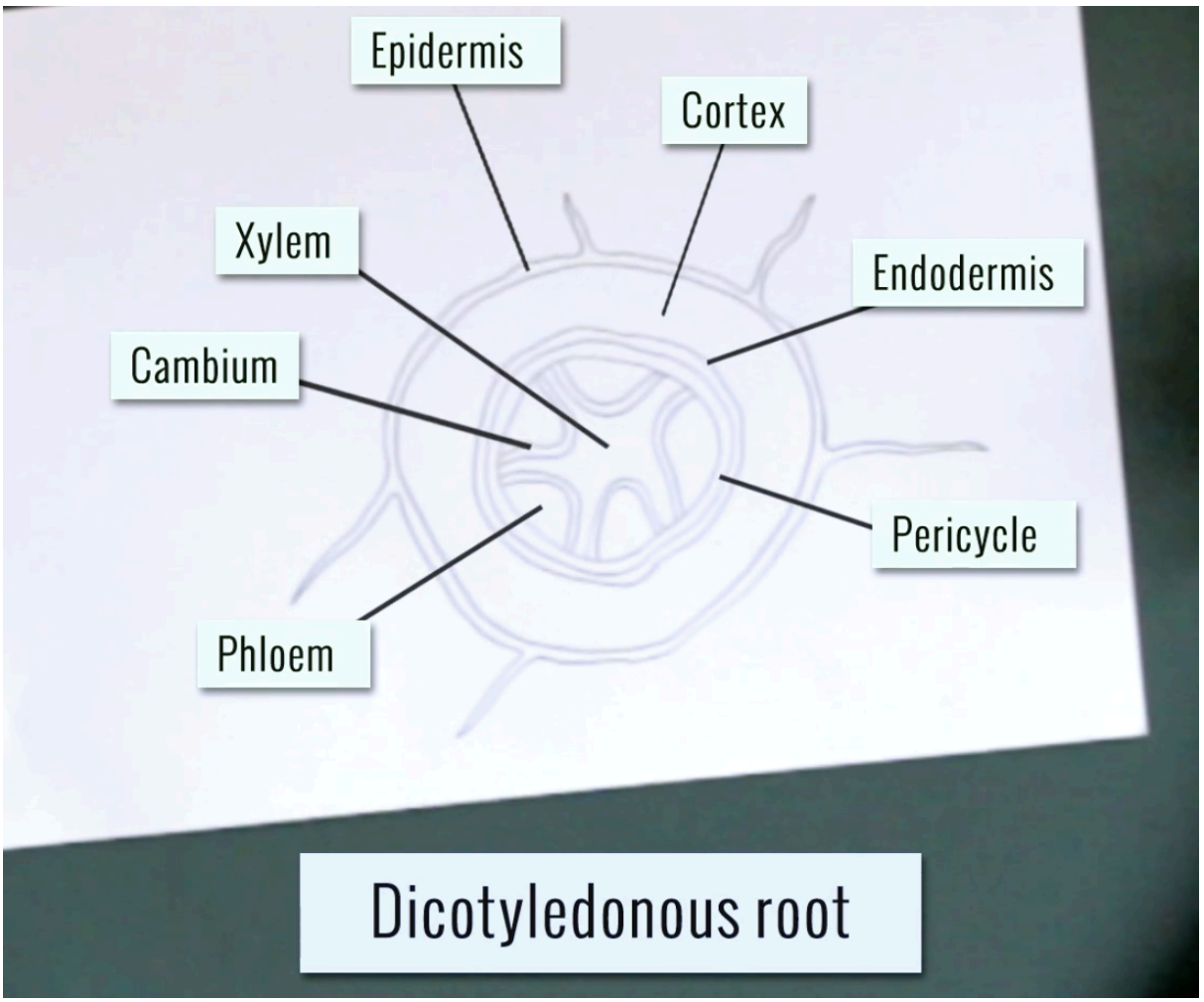
2. Size of drawing
    - Use up most of the space provided
    - BIG!!

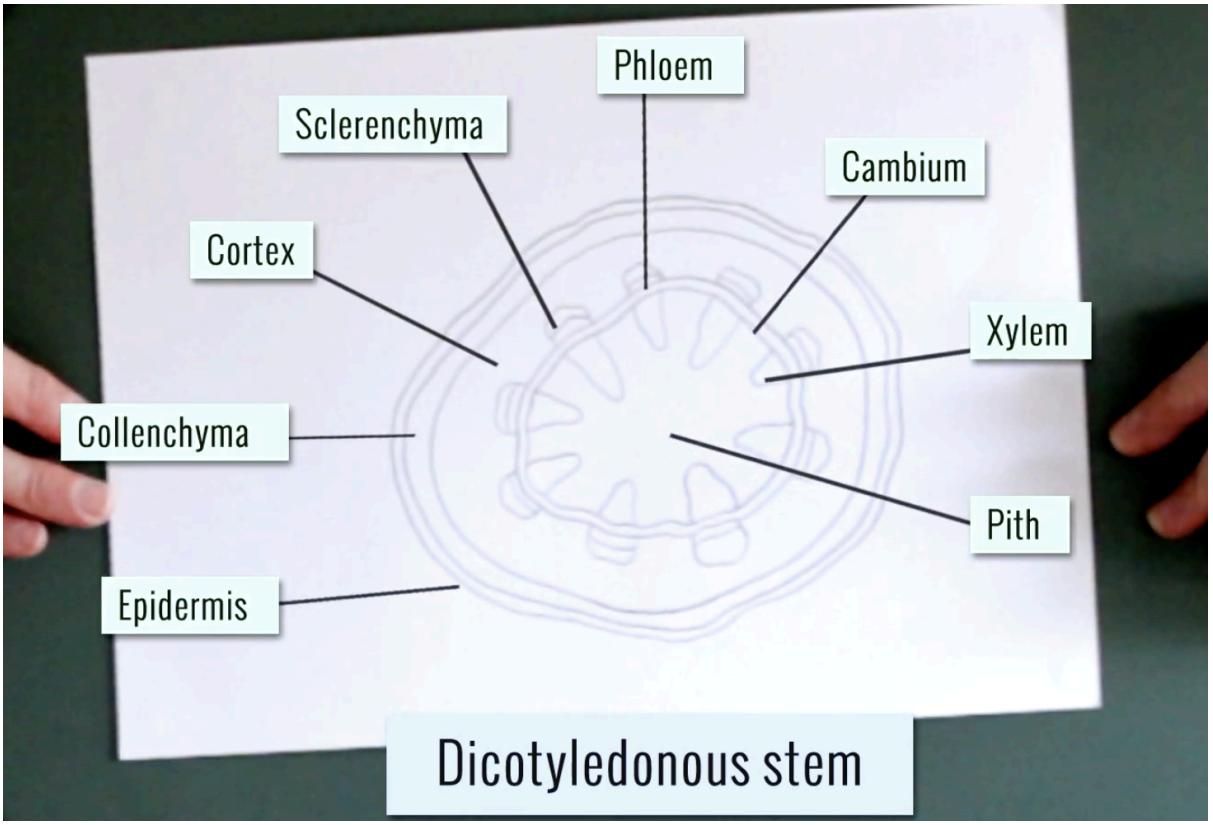
3. Number of layers
    - Use low power objective lens while drawing diagram
    - Use high power to double check
    - If you’re not sure how many layers, draw more to be on the safer side

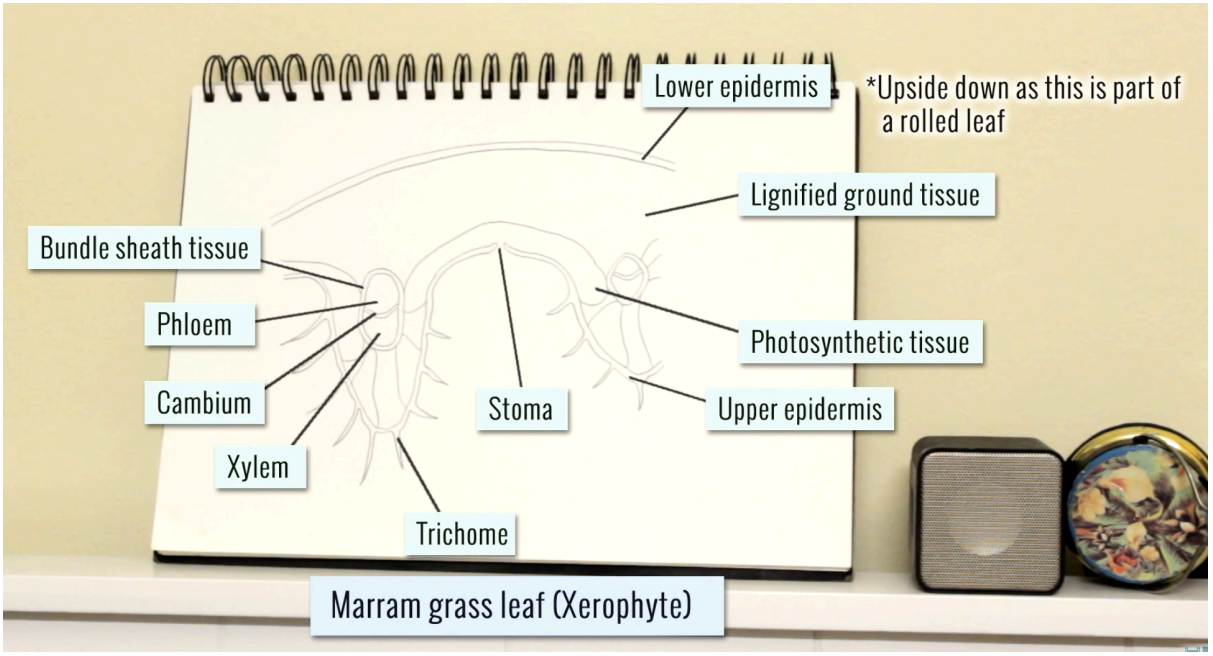
4. Relative thickness of layers
    - Use eyepiece graticule/ ruler to compare thickness of layers

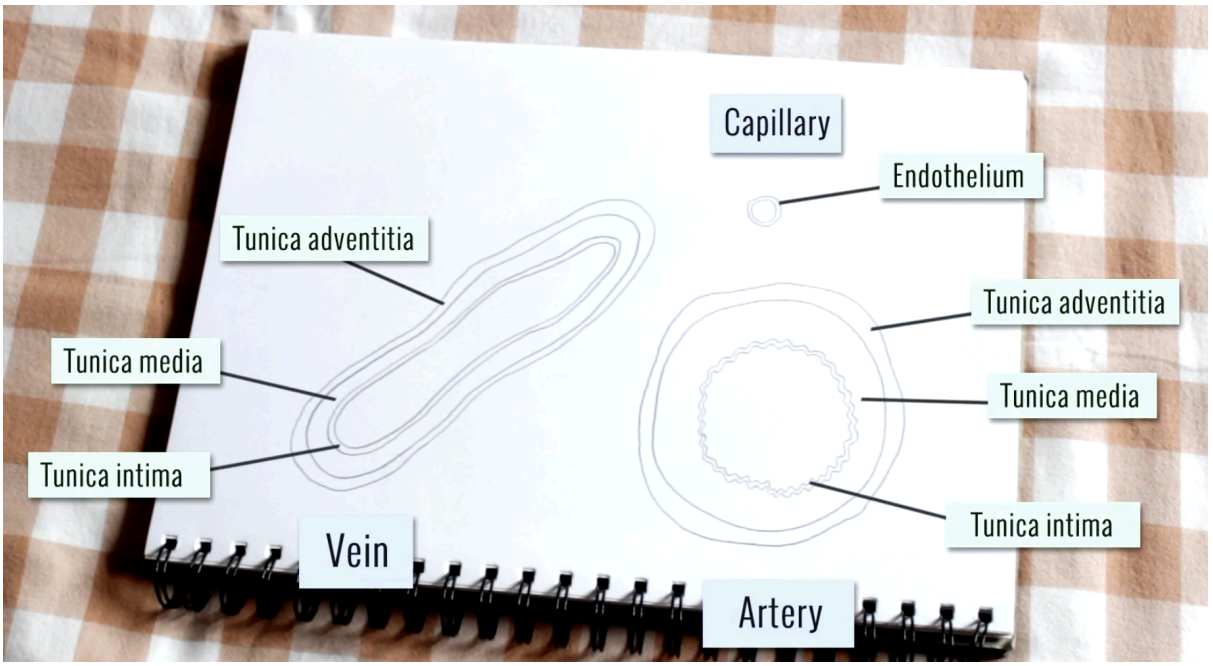
5. Labels
    - Always use ruled label line
    - One label line for one name
    - Label line should be in between 2 lines representing the layer
    - Only label whatever you are asked to

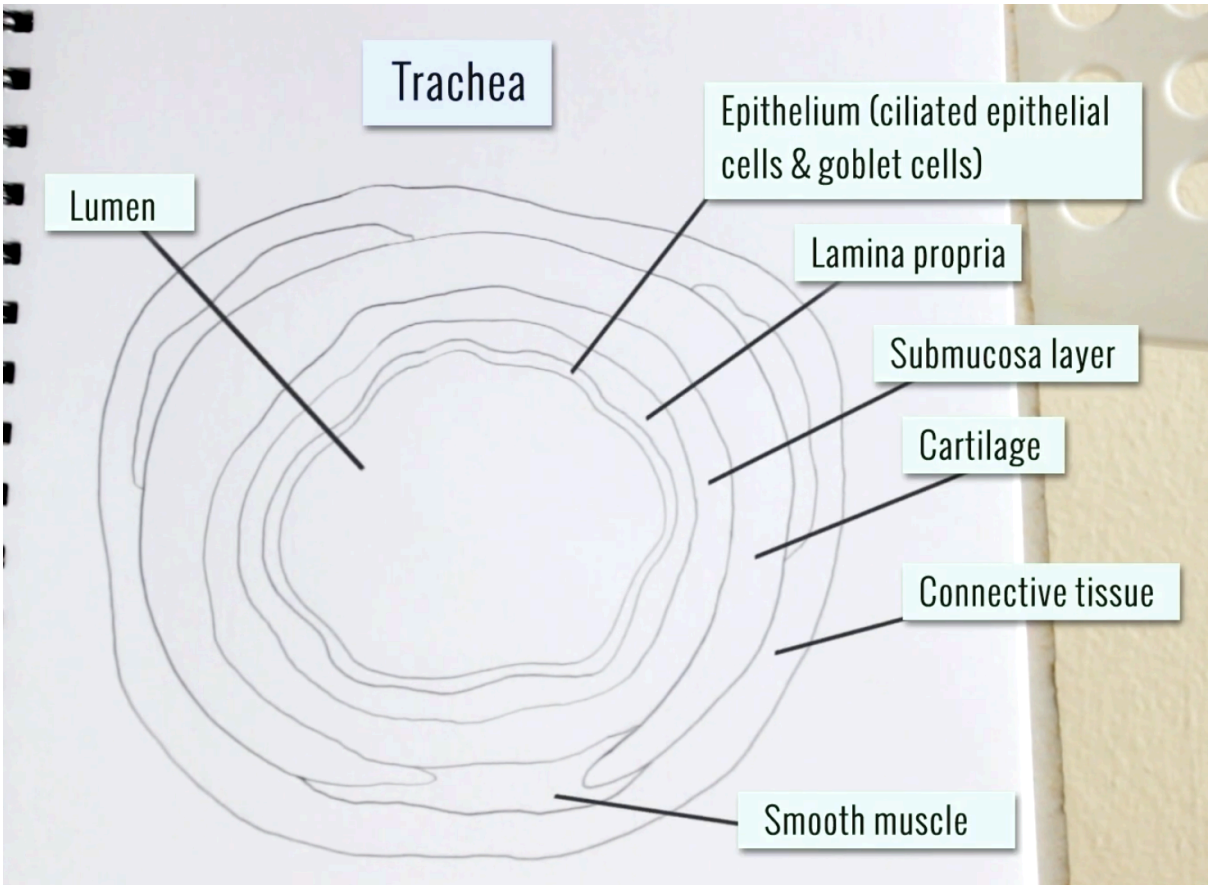


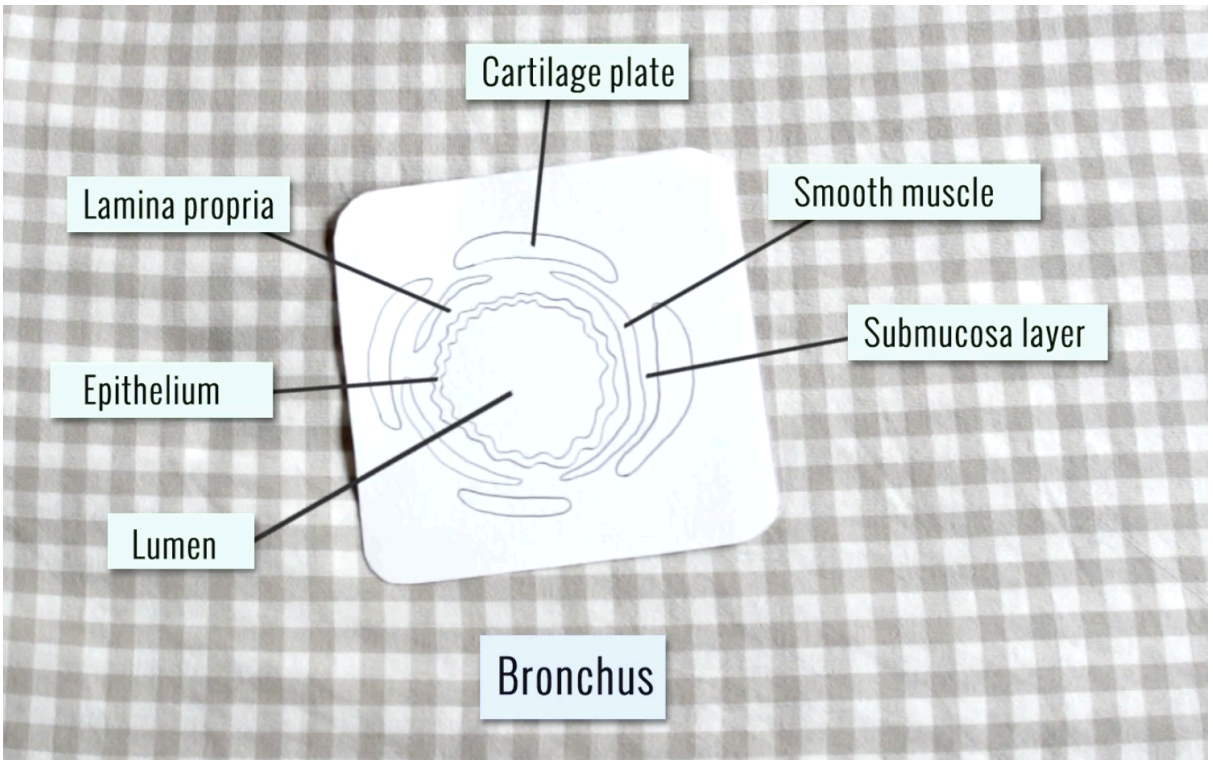

Epidermis
Cortex
Xylem
Endodermis
Cambium
Pericycle
Phloem
Dicotyledonous root

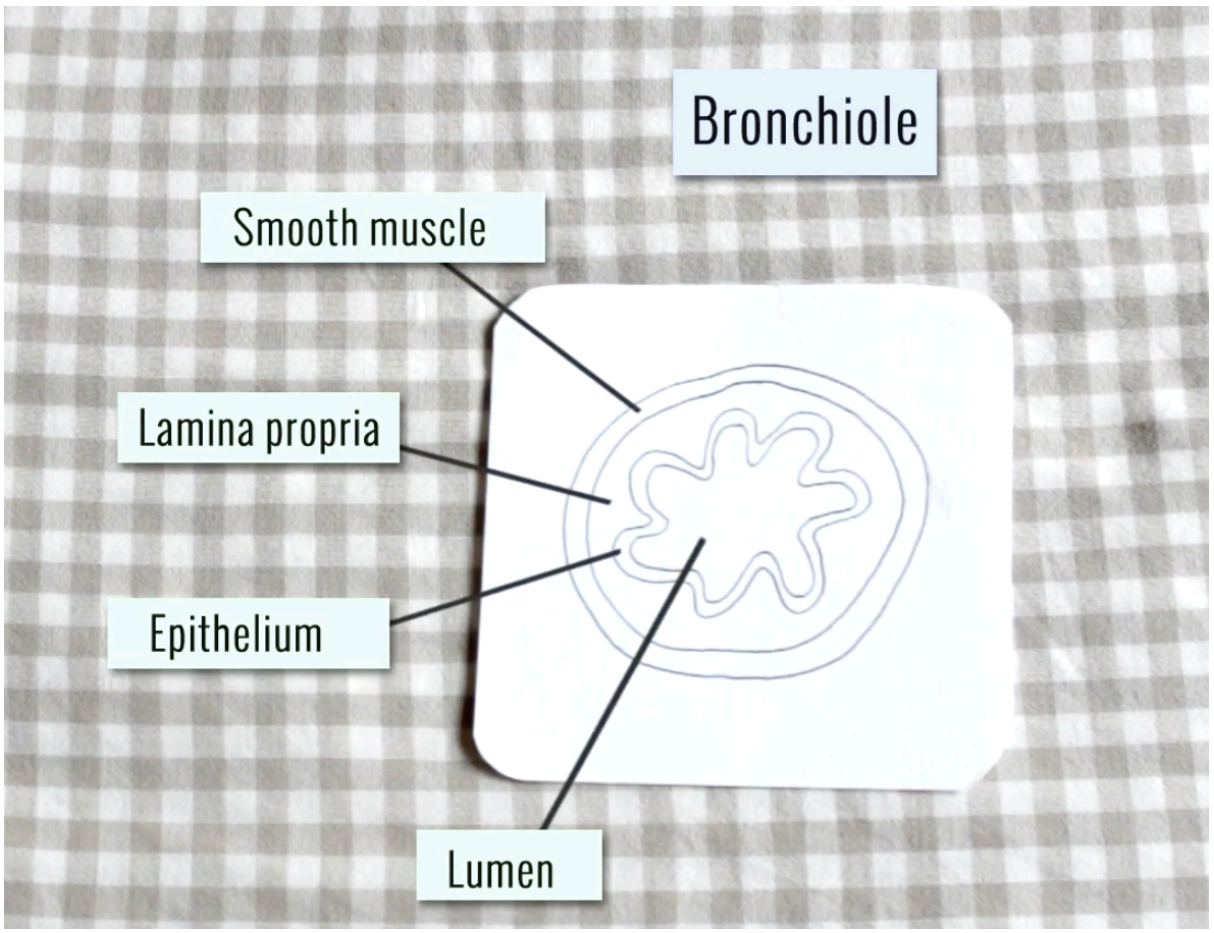

Phloem
Sclerenchyma
Cambium
Cortex
Xylem
Collenchyma
Pith
Epidermis
Dicotyledonous stem

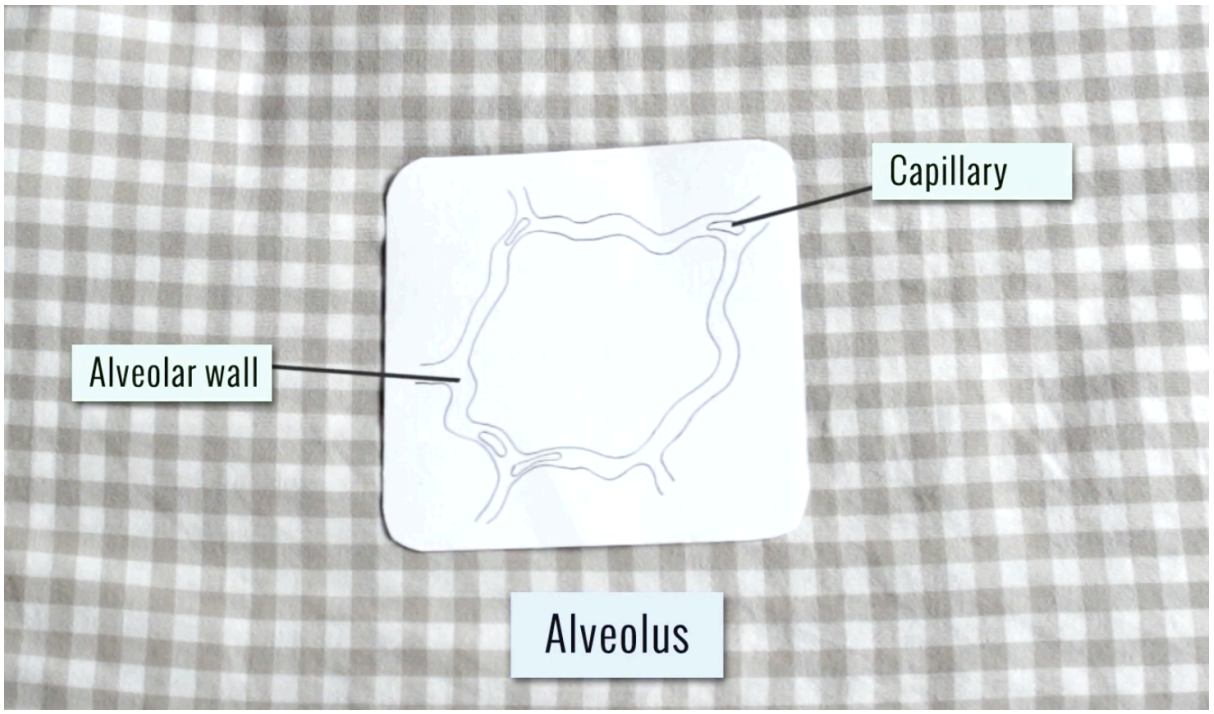

Lower epidermis
*Upside down as this is part of a rolled leaf
Lignified ground tissue
Bundle sheath tissue
Phloem
Cambium
Xylem
Trichome
Stoma
Photosynthetic tissue
Upper epidermis
Marram grass leaf (Xerophyte)


Capillary
Endothelium
Tunica adventitia
Tunica media
Tunica intima
Vein
Tunica adventitia
Tunica media
Tunica intima
Artery


Trachea
Epithelium (ciliated epithelial cells & goblet cells)
Lumen
Lamina propria
Submucosa layer
Cartilage
Connective tissue
Smooth muscle


Cartilage plate
Lamina propria
Smooth muscle
Submucosa layer
Epithelium
Lumen
Bronchus





Detailed drawings

Shows cells and its visible structures

1. Quality of drawing
    - Clear, sharp, fine, unbroken lines
    - Not ruled
    - No feathery lines & dashed lines

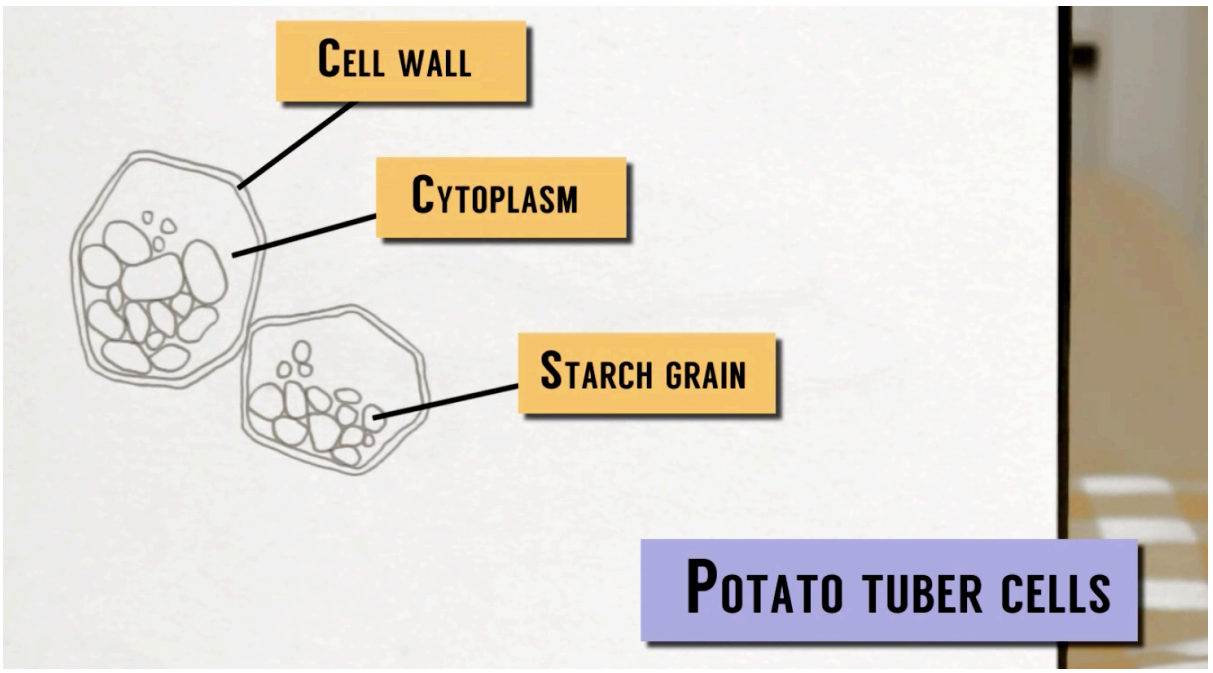
2. Follow instructions
    - Draw exact number of cells asked
    - If asked to draw adjacent cells, make sure they are touching; They should not overlap!
    - If 2 adjacent plant cells, there should be 3 lines between the 2 adjacent cells; middle line = middle lamella

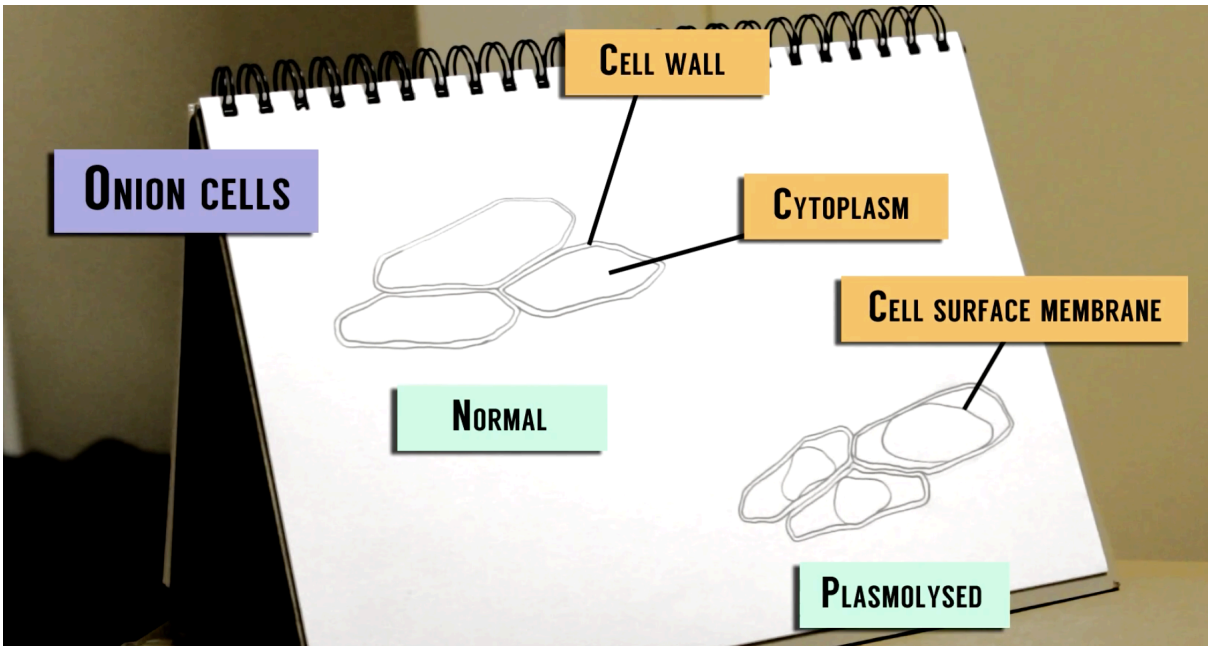
3. Size of drawing
    - Use up most of the space provided
    - BIG!!

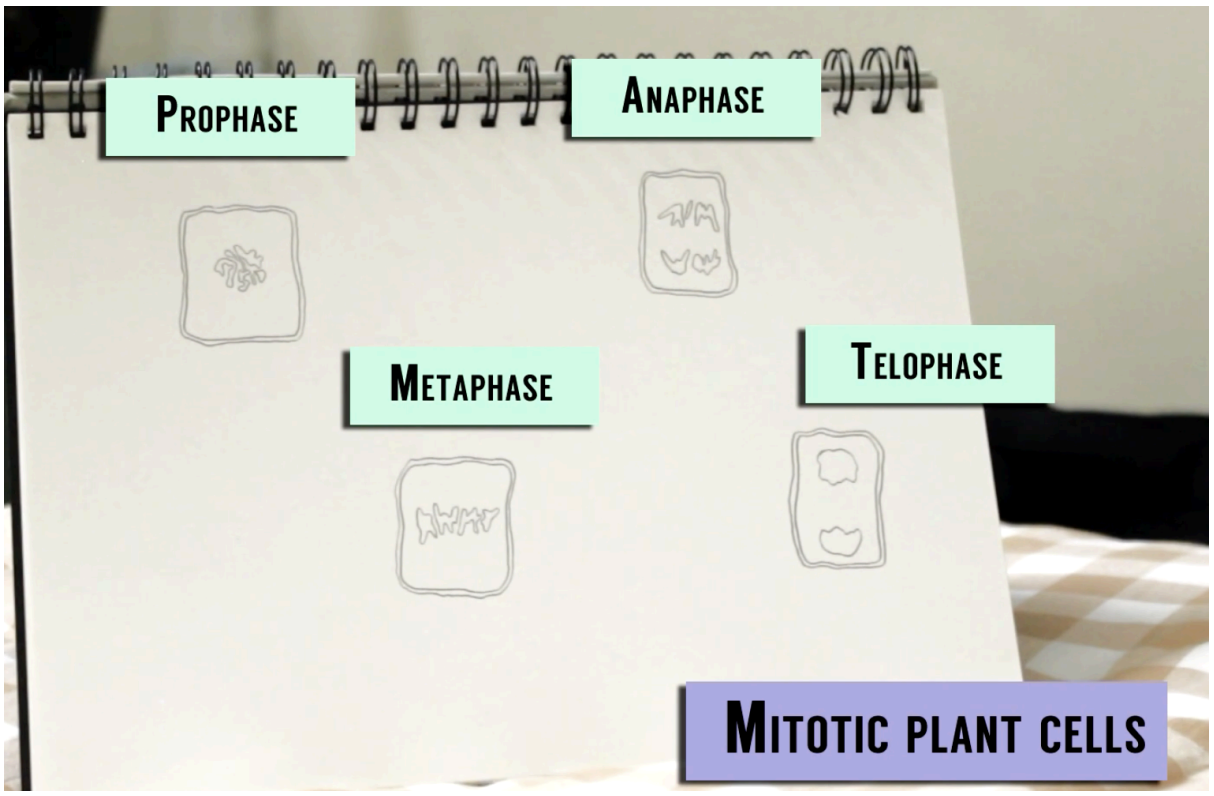
4. Show variations
    - Cells are not identical
    - Spot small differences between them

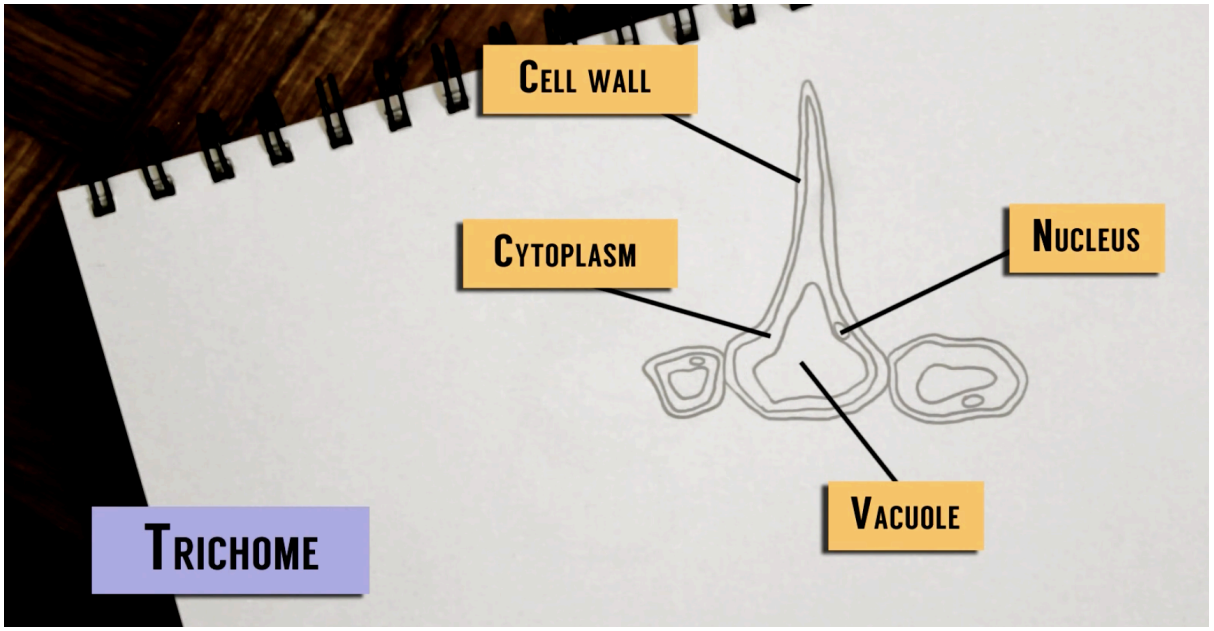
5. Relative size of different structures
    - Proportion of different cell structures should be shown correctly

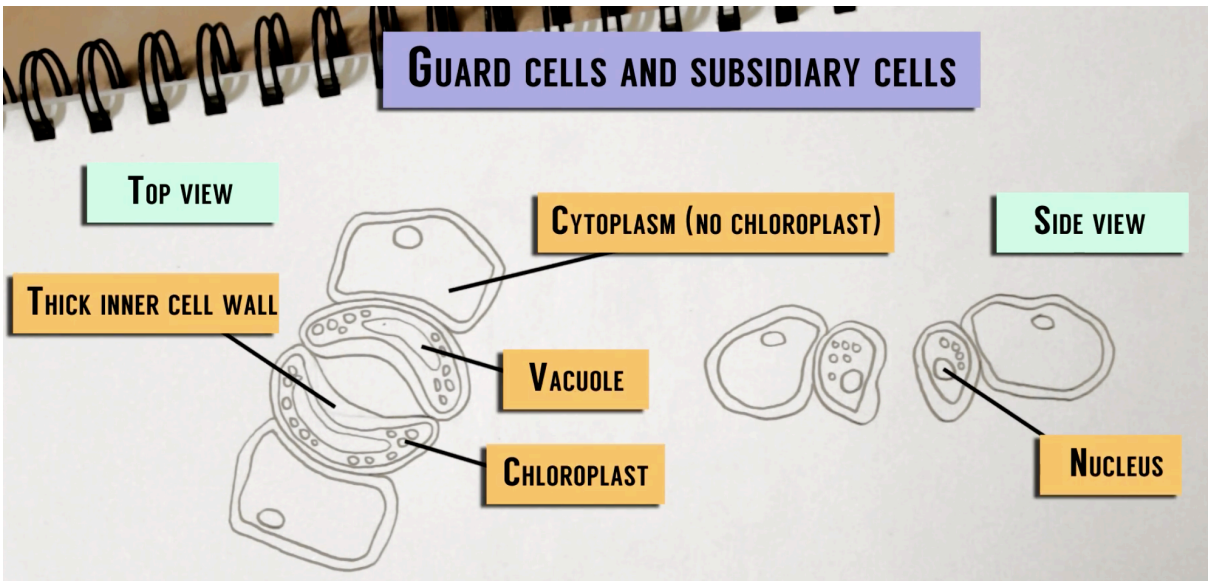
6. Label
    - Only when asked to
    - Use a ruled label line
    - Line should touch the structure
    - 1 line to 1 label

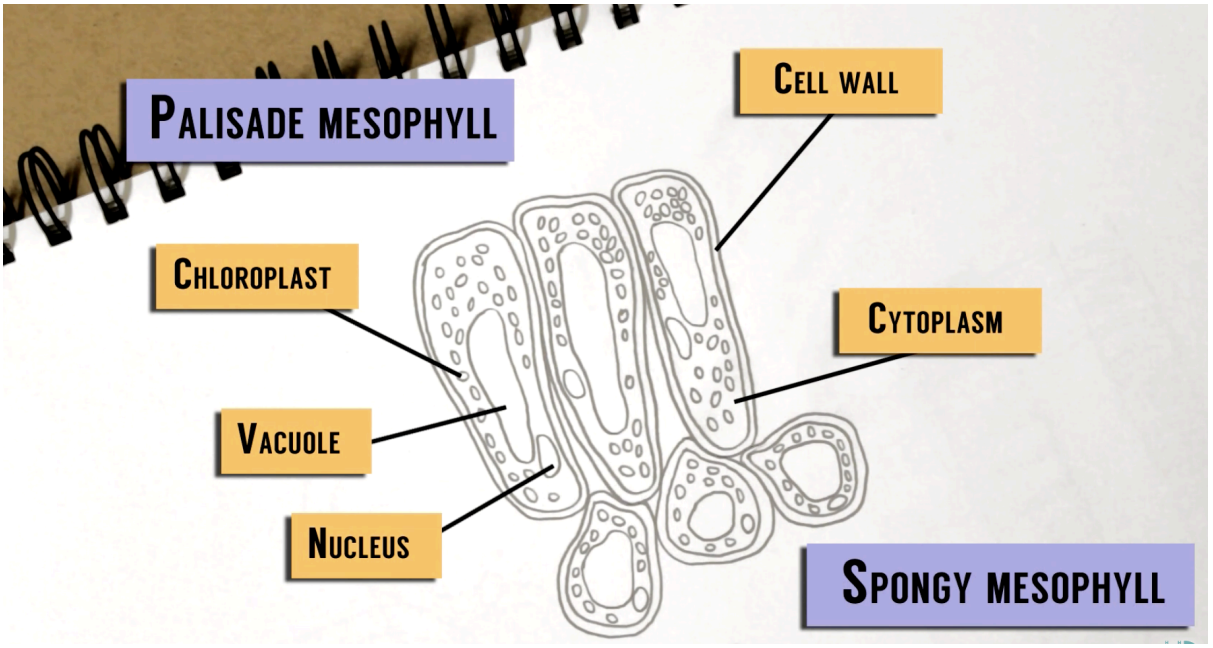

Cell wall
Cytoplasm
Starch grain
Potato tuber cells

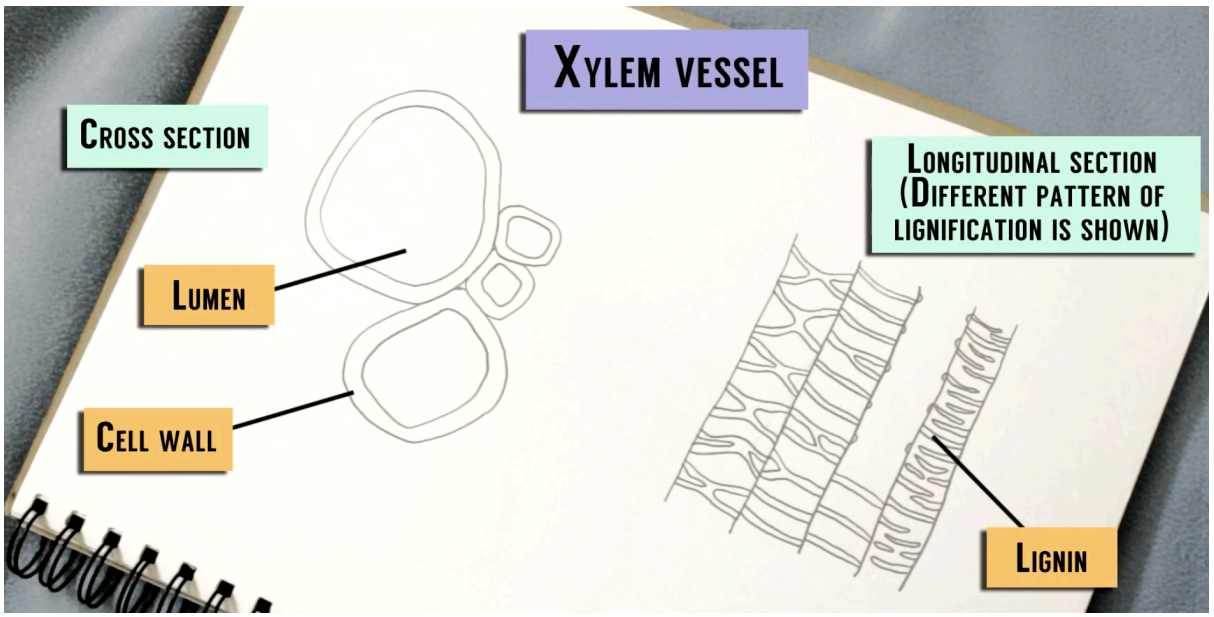

Onion cells
Cell wall
Cytoplasm
Cell surface membrane
Normal
Plasmolysed

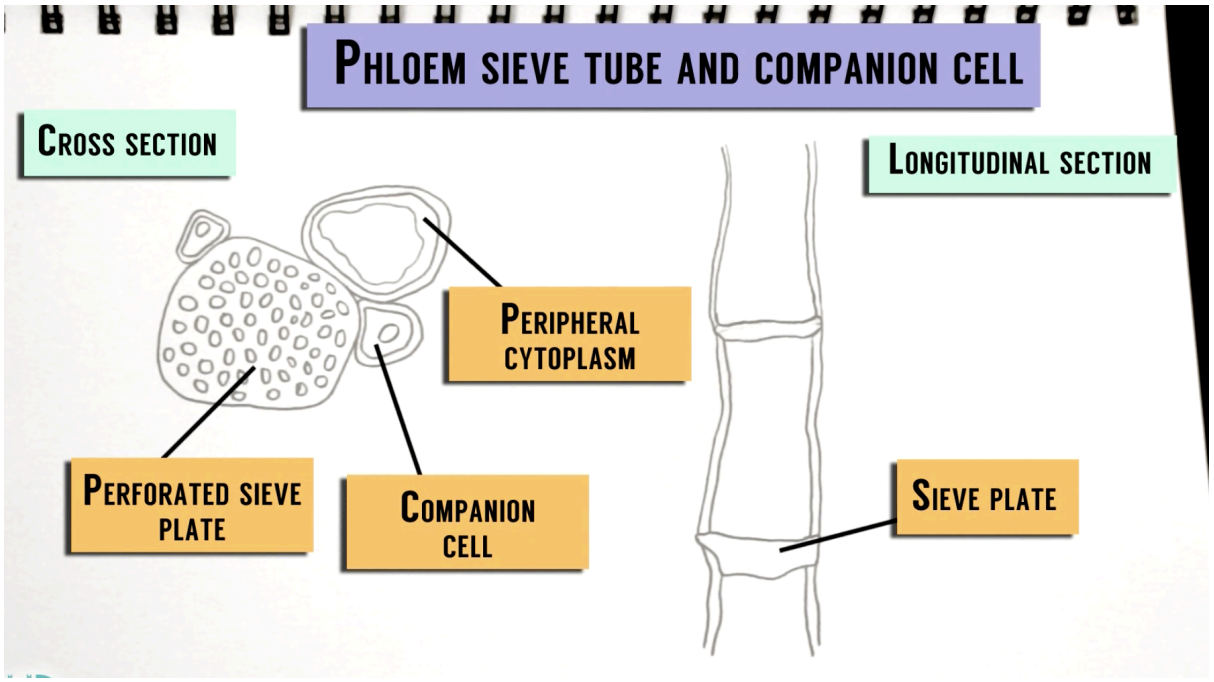

PROPHASE
ANAPHASE
METAPHASE
TELOPHASE
MITOTIC PLANT CELLS

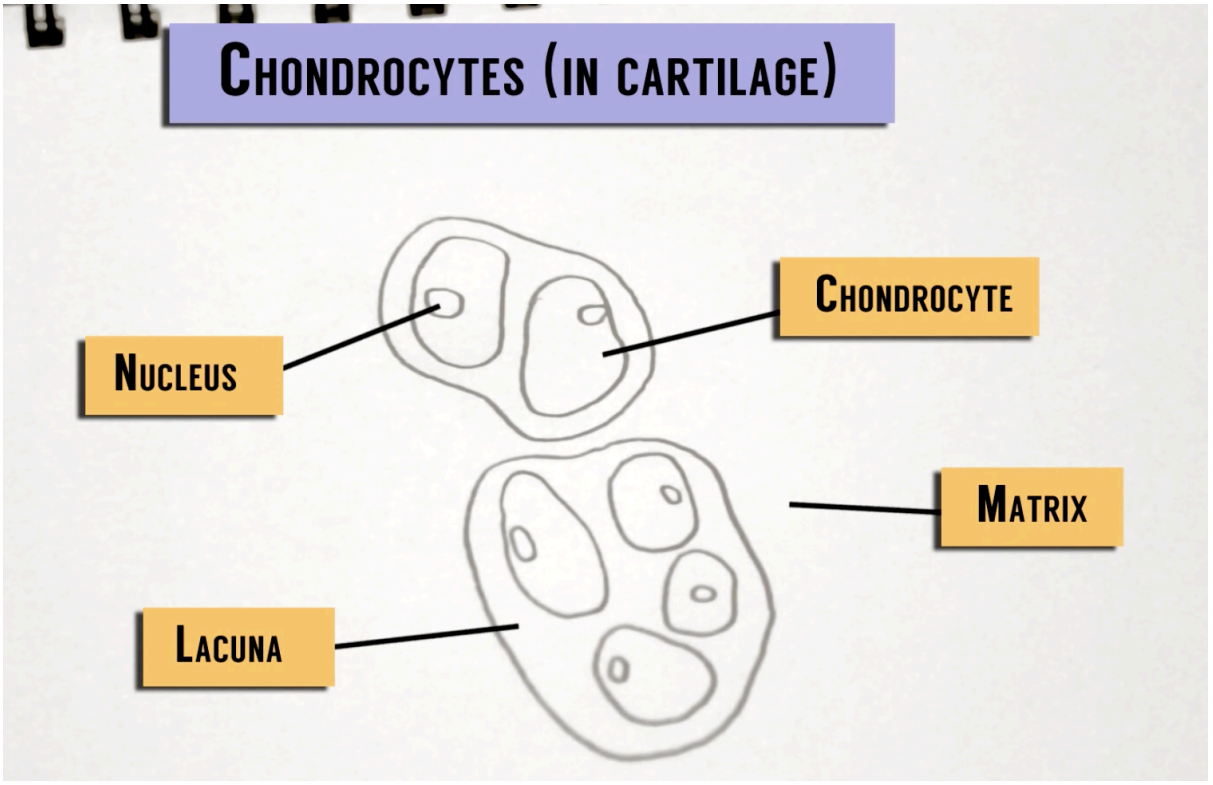

CELL WALL
CYTOPLASM
NUCLEUS
VACUOLE
TRICHOME

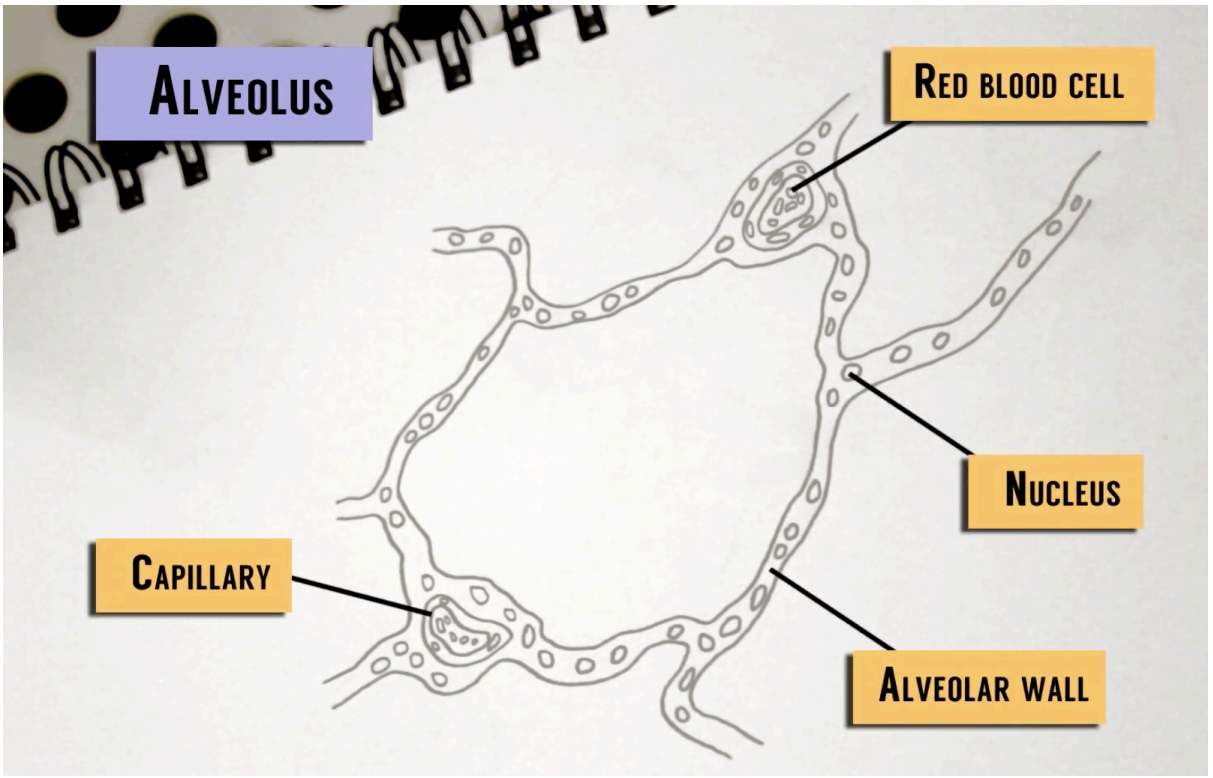

GUARD CELLS AND SUBSIDIARY CELLS
TOP VIEW
CYTOPLASM (NO CHLOROPLAST)
SIDE VIEW
THICK INNER CELL WALL
VACUOLE
CHLOROPLAST
NUCLEUS

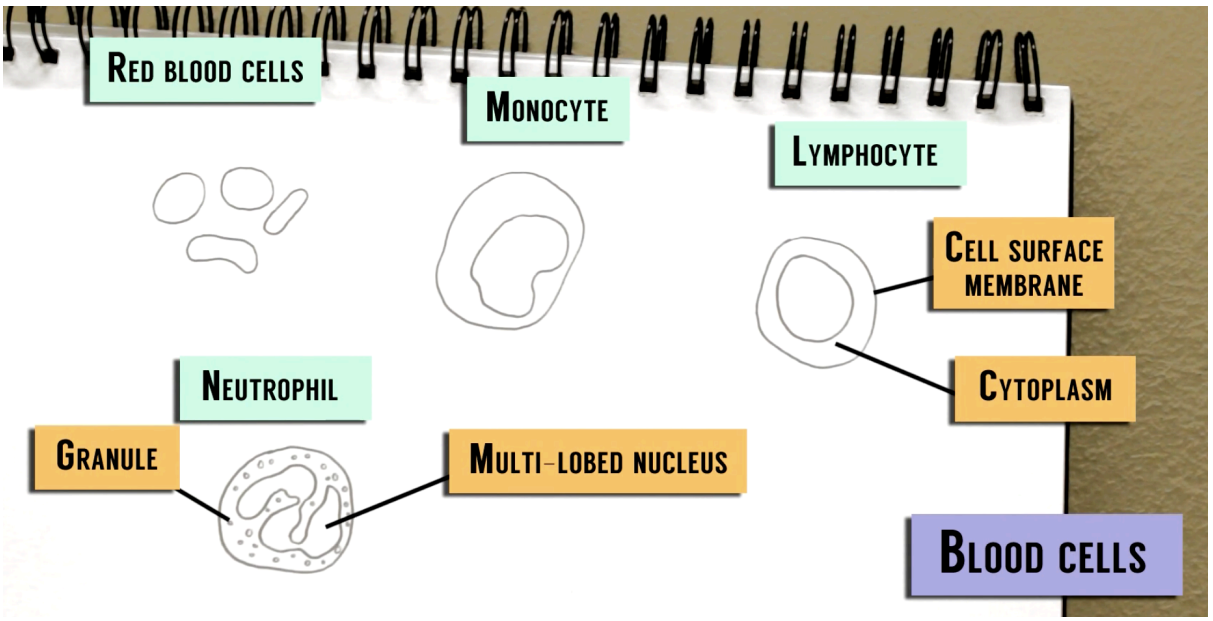

PALISADE MESOPHYLL
CELL WALL
CHLOROPLAST
CYTOPLASM
VACUOLE
NUCLEUS
SPONGY MESOPHYLL

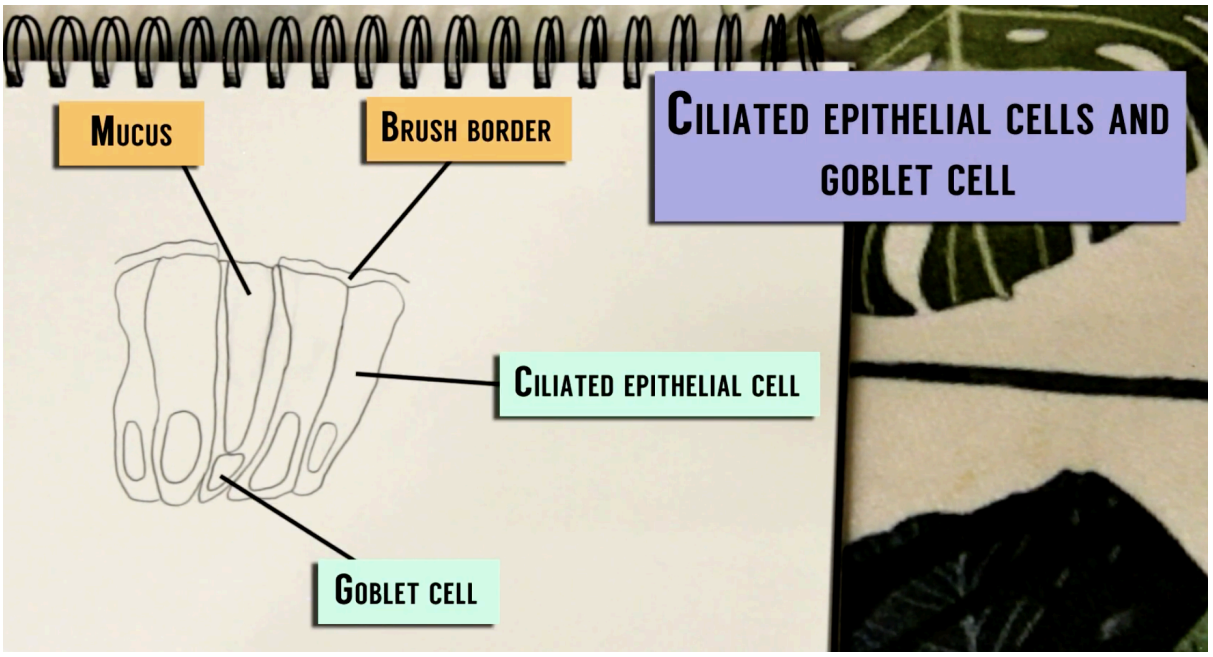

XYLEM VESSEL
CROSS SECTION
LONGITUDINAL SECTION (DIFFERENT PATTERN OF LIGNIFICATION IS SHOWN)
LUMEN
CELL WALL
LIGNIN

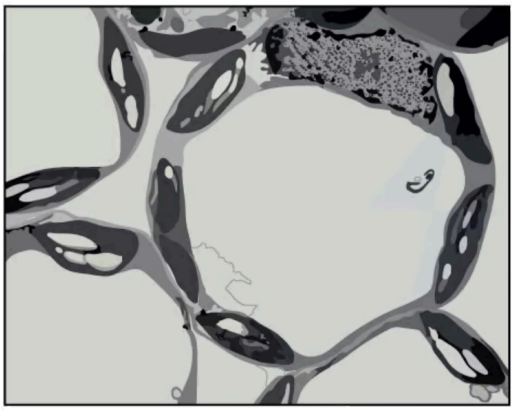

PHLOEM SIEVE TUBE AND COMPANION CELL
CROSS SECTION
LONGITUDINAL SECTION
PERIPHERAL CYTOPLASM
PERFORATED SIEVE PLATE
COMPANION CELL
SIEVE PLATE

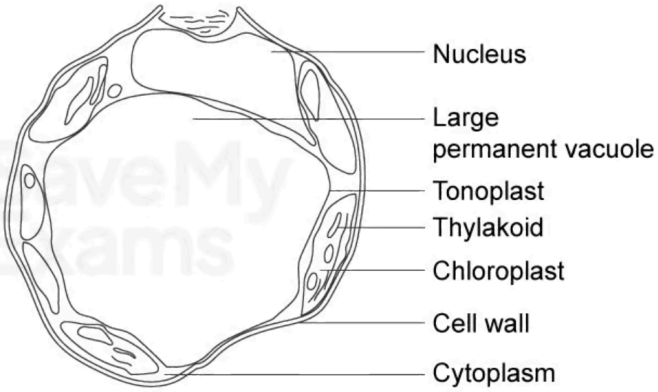

Chondrocytes (in cartilage)
Nucleus
Chondrocyte
Matrix
Lacuna

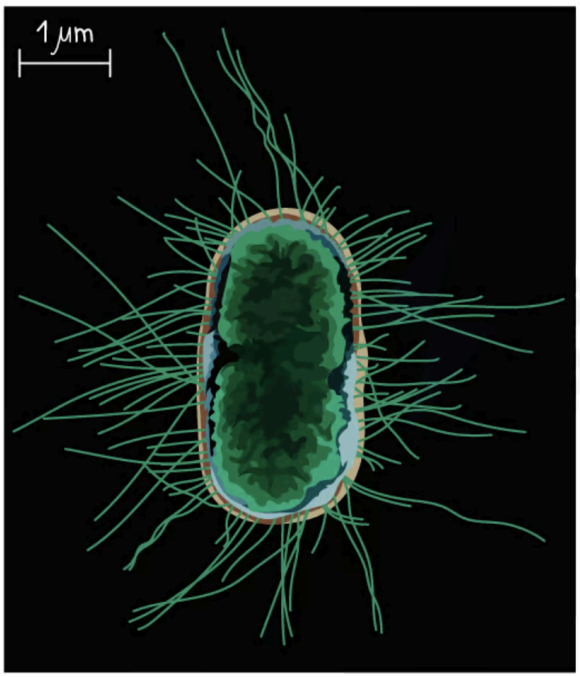

Alveolus
Red blood cell
Nucleus
Capillary
Alveolar wall

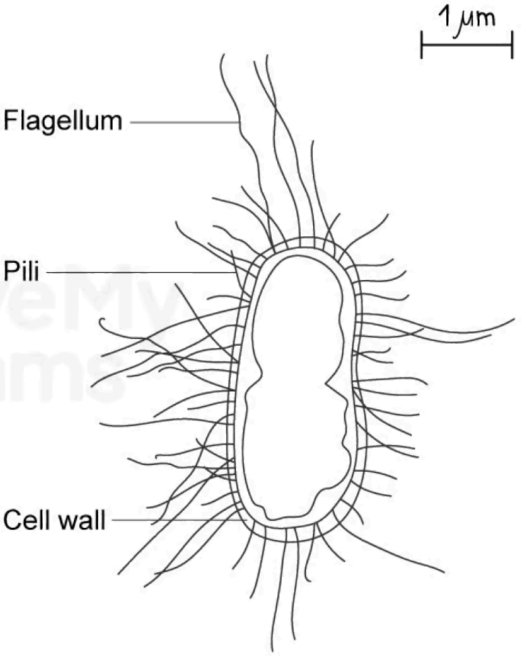

Red blood cells
Monocyte
Lymphocyte
Cell surface membrane
Cytoplasm
Neutrophil
Granule
Multi-lobed nucleus
Blood cells

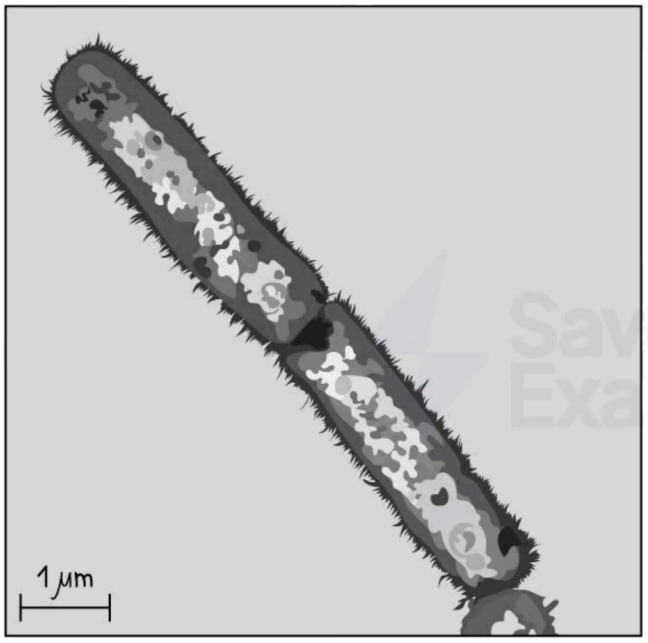

Mucus
Brush border
Ciliated epithelial cells and goblet cell
Ciliated epithelial cell
Goblet cell

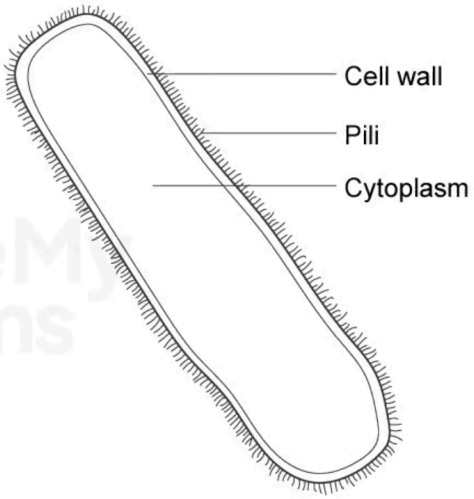


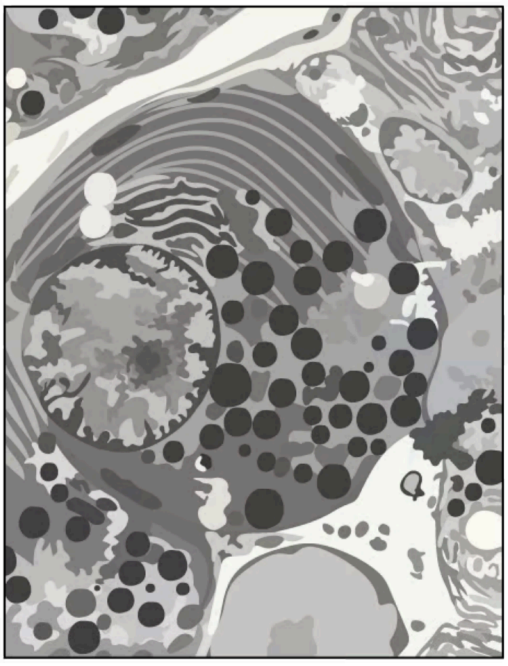

Nucleus
Large permanent vacuole
Tonoplast
Thylakoid
Chloroplast
Cell wall
Cytoplasm

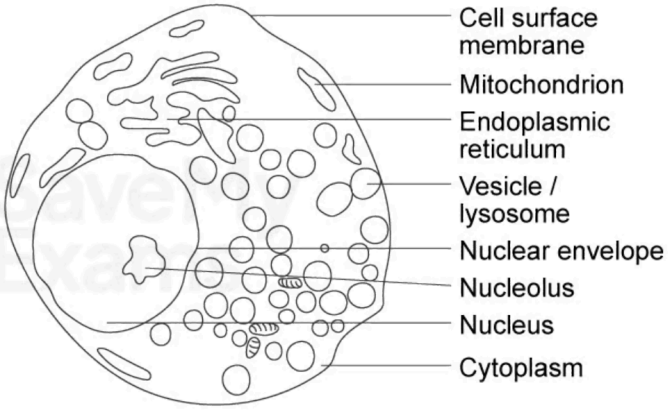

1 μm


1 μm
Flagellum
Pili
Cell wall


1 μm


Cell wall
Pili
Cytoplasm




Cell surface membrane
Mitochondrion
Endoplasmic reticulum
Vesicle / lysosome
Nuclear envelope
Nucleolus
Nucleus
Cytoplasm