Cambridge Assessment International Education

Teaching Pack

Investigating the effect of carbon dioxide concentration on stomatal density

Cambridge International AS & A Level Biology 9700

Version 1.0

Cambridge Pathway

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Introduction

This pack will help you to develop your learners' experimental skills as defined by assessment objective 3 (AO3 Experimental skills and investigations) in the course syllabus.

Important note
Our *Teaching Packs* have been written by **classroom teachers** to help you deliver topics and skills that can be challenging. Use these materials to supplement your teaching and engage your learners. You can also use them to help you create lesson plans for other experiments.

This content is designed to give you and your learners the chance to explore practical skills. It is not intended as specific practice for Paper 3 (Advanced Practical Skills) or Paper 5 (Planning, Analysis and Evaluation).

This is one of a range of *Teaching Packs* and each pack is based on one experiment. The packs can be used in any order to suit your teaching sequence.

The structure is as follows:

Briefing lesson (1 hour*)
This lesson reinforces the key concepts, relevant skills, knowledge and understanding linked to the experiment.

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Planning lesson (1 hour*)
This lesson focuses on planning an experiment.
It ends with a demonstration video of the experiment.

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Lab lesson (1 hour*)
This lesson focuses on carrying out the experiment including the collection and recording of observations, measurements and estimates.

↓

Debriefing lesson (1 hour*)
This lesson focuses on the analysis and interpretation of data.
This includes making conclusions, evaluating methods and the quality of data, and how improvements could be made.

** the timings are a guide only; you may need to adapt the lessons to suit your circumstances.*

In this pack you will find lesson plans, worksheets and teacher resource sheets.

Experiment: Investigating the effect of carbon dioxide concentration on stomatal density

This *Teaching Pack* focuses on an investigation of the impact of environmental factors on stomatal density.

Stomata control the movement of gases into and out of a leaf. In this experiment, your learners will use microscopy to find the stomatal density of plant leaves grown in high, atmospheric and low carbon dioxide concentrations.

This experiment has links to the following syllabus content (see syllabus for detail):

- 7.2 Transport mechanisms
- 14.2 Homeostasis in plants

The experiment covers the following experimental skills, as listed in **AO3: Experimental skills and investigations:**

- plan experiments and investigations
- collect, record and present observations, measurements and estimates
- analyse and interpret data to reach conclusions
- evaluate methods and quality of data and suggest improvements.

The following techniques are used:

- measuring by counting squares in a grid to estimate surface area
- measuring by counting numbers of cells
- setting up and using a light microscope to view and observe specimens
- correctly identifying cells using a light microscope
- staining and preparing a slide of cells
- estimating the number of cells in a field of view using a sample
- (optional) calibrating an eyepiece graticule using a stage micrometer.

Prior knowledge

Knowledge from the following syllabus topics is useful for this experiment.

- 1.1 The microscope in cell studies
- 18.2 Biodiversity
- 7.1 Structure of transport tissues

Briefing lesson: Sampling

Resources	• Worksheets A, B and C • Teacher Instructions 1
Learning objectives	By the end of the lesson: • ***all*** learners should know what stomatal density is and its units • ***most*** learners should understand the need for both a random and a representative sample • ***some*** learners will be able to use a running mean to ensure a representative sample.

Before the lesson, photocopy Teacher Instructions 1 onto sheets of acetate, enough for each pair of learners to be given one 3 cm^2 piece containing a circle with diameter of 2 cm.

Timings	Activity
10 min	**Starter/Introduction** Give your learners Worksheet A. Explain that the photo shows part of a leaf surface as seen under a light microscope. Ask your learners what the structures are. With some prompting, they should recognise stomata. Learners should add labels to show one set of guard cells and a pore (stoma); ask some volunteers to briefly explain the function of the stomata and the guard cells. Ask learners what they think 'stomatal density' means. If necessary, encourage them to use what they can see on Worksheet A to help. Check that they understand that it is the number of stomata per unit area and that the appropriate units are 'number of stomata / field of view' or 'number of stomata / mm^2'. Ask pairs of learners to consider the environmental factors that might affect stomatal density. Ask them to suggest what the impact might be and what this would mean for the plant in terms of transpiration. The environmental factors that might affect the stomatal density of a plant are likely to be the same as those that affect its transpiration rate. These could include the light intensity a plant is exposed to (sunlight or shade); the mean air temperature; the mean humidity; or water and CO_2 availability. For example, some xerophytic plants are adapted to dry conditions by having a low stomatal density.
10 min	**Main lesson** Ask learners how they might find the stomatal density of a leaf with the units 'number of stomata / field of view'. With help, they should be able to deduce viewing the leaf under a light microscope and counting the number of stomata in a field of view. In groups of 2–4, ask learners to discuss how they might find the area of leaf surface visible in the field of view, in order to calculate the stomatal density with units 'number of stomata / mm^2'. Possible suggestions include viewing a graph paper grid with the same lens and counting squares visible in the field of view. Alternatively, with prompting, they might recall using an eyepiece graticule and suggest using this to measure the diameter of the field of view. With the diameter of the field of view they can calculate the area using πr^2.

Timings	Activity
	Ask learners to calculate the area of the field of view on Worksheet B and to find the stomatal density for the specimen shown. In the same groups of 2–4, give each learner Worksheet C and the 3 cm^2 piece of acetate prepared earlier. Explain that the circle represents the field of view of a microscope. Learners should place the circle onto Worksheet C and count the number of stomata within the circle. Ask them if this is a representative value for the whole area. Discuss the inaccuracy caused by placing the circle on a cluster of stomata or where there are fewer stomata towards the periphery. Ask your learners to devise a method to obtain a stomatal count that is more representative of the whole area. Ask them to share their ideas with the class and discuss the advantages and disadvantages. They might suggest multiple samples but might need prompting to suggest that an adequate sample number is reached when the mean count becomes stable. They might also need prompting to suggest random positioning of the sample by, for example, drawing gridlines onto Worksheet C and generating random coordinates. Discuss the importance of the sample being chosen at random. Some suggested methods are provided in the answers to Worksheet C. Discuss what is meant by 'adequate accuracy' in this context (counting all the stomata would give the most accurate count but would be time-consuming, so using a running mean to find the sample size when the mean count becomes stable should provide a good balance between accuracy and time taken). Ask your learners to carry out their method to find a representative mean count using a series of random samples.
	**Plenary** Ask learners to suggest how randomisation of the position of the field of view could be achieved on a microscope. Suggestions might include random movements of the slide; fixing graph paper scales to the stage; or using a mechanical stage and moving the slide by random increments.

Planning lesson: Detecting a difference

Resources	• Worksheet D • *Investigating the effect of carbon dioxide …*video
Learning objectives	By the end of the lesson: • ***all*** learners should understand that the experiment aims to find any differences in the plants after the six weeks • ***most*** learners should understand the need for initial and final data for plants kept in different carbon dioxide concentrations • ***some*** learners will recognise that a statistical test is required to see if any differences are significant.

Timings	Activity
15 min	**Starter/Introduction** Ask learners to draw a concept map to show what makes a good plan for a scientific investigation. Allow some initial thinking and writing time and then invite learners to offer suggestions and discuss as a class; they can then add to and edit their concept maps during the discussion. The concept map should include: 'identifying what needs to be investigated'; 'identifying the variables'; 'identifying safety requirements'; 'considering what apparatus is needed'; 'deciding the measurement/repeats required'; 'stating a hypothesis'; 'deciding the technique(s) to use'. For each element of the map, ensure the discussion goes into sufficient depth. For example, if a learner mentions 'identifying the variables', guide the discussion into what sort of variables there are (independent, dependent, standardised) and which ones they should change / keep the same / measure. Point out the links between all the considerations, for example, the variable you plan to measure will determine the apparatus you need to use.
30 min	**Main lesson** Tell your learners that they are going to plan an experiment to investigate the effect of long-term altered carbon dioxide concentration on stomatal density. They should use their concept maps, Worksheet D and the discussions from the *Briefing lesson* to help them. The main focus of their plan is to devise a method to detect any change in stomatal density after a plant has spent six weeks exposed to either a raised or a lowered carbon dioxide concentration. Learners are not expected to know all the techniques required (these are provided on Worksheet D) but their plan should explain how to put them all together along with a way to sample the plants for stomatal density. Before they begin, have discussions to cover some of the aspects they might not be familiar with. For instance, ask them if they should use young (new) leaves or old (large) leaves when measuring stomatal density and why; why they can't create a range of carbon dioxide concentrations in the school laboratory; why hydrochloric acid is dripped onto marble chips and why soda lime is used. Make sure learners understand that the existing (older) leaves detect the change in

Timings	Activity
	carbon dioxide in the atmosphere and the plant responds by developing new leaves with a different stomatal density; the stomatal density on the existing leaves cannot change. Therefore, which leaves learners select for sampling is really important. They should describe both the apparatus and any safety precautions. Encourage learners to justify each part of their method. You may need to prompt learners during the writing of their plans. In particular, ensure that they understand that they need both initial and final data and that the leaves they sample must be standardised as far as possible. Remind them of the sampling techniques they have looked at so far. They should also be asked how they will determine if any detected differences after six weeks are significant; learners might suggest using values of standard deviation or standard error, or graphs including standard error bars; some learners might suggest that they need to use a statistical test. A 'model' plan is provided in the answers to Worksheet D. Remind learners to describe the steps they will take in sufficient detail that another person could follow their plan.
	**Plenary** Learners watch the '*Investigating the effect of carbon dioxide concentration on stomatal density'* video as a summary of the experiment. Ask your learners to identify any key points they have missed out of their own procedure. For example, it is likely that some learners will not have recognised the need for standardising the age of the leaves (by standardising the position from which they picked the leaf, for example, third leaf down from apex) or will not have included a way of ensuring a sufficient number of samples for each leaf. Ask them to think of things that might go wrong, such as mixing up samples, and how they could avoid this (labelling tiles/slides ahead of time and placing the sample immediately on them). Give learners some time to make adjustments to their plan. They will use their plan in the *Lab lesson*, so make sure you're happy with what they will follow.

Lab lesson: Comparing stomatal densities

Resources	• *Teacher walkthrough* video • Worksheets B and E • Teacher Instructions 2 • Equipment as per *Teacher notes* • Initial data to supply to learners
Learning objectives	By the end of the lesson: • ***all*** learners should be able to sample each plant and record some results in a suitable table • ***most*** learners should be able to take steps to standardise the leaves they use and record a full set of accurate results • ***some*** learners will express stomatal density as the number of stomata / mm^2 by finding the area of the field of view.

Note: you should have been growing the three plants in the appropriate conditions for six weeks ***prior*** to the start of this lesson. See Teacher instructions 2 for details.

Timings	Activity
10 min	**Starter/Introduction** Have a discussion about what a results table should look like; agree as a class what its key features should be. Suggestions might include fully ruled columns and rows; independent variable in the left column or top row; clear headings with appropriate units; no units in the body of the table; data recorded to a number of significant figures appropriate to the measuring instrument used. In this experiment, the table needs space for initial and final raw data. Ask learners to create a suitable results table – supply them with the initial data to record (you should have already collected the data yourself from the plants that will be used in the *Lab lesson*, **six weeks** prior to the start of this lesson.)
10 min 30 min	**Main lesson** Explain that the three plants have already been grown in the sealed (air-tight) containers for six weeks and their initial stomatal densities calculated. They just need to calculate the stomatal densitites for the *after* data. Learners should collect their equipment and set up their microscopes (and graticules if they are using them). Support for setting up and using a light microscope can be found on Worksheet E. (Depending on your learners, you might decide that it would be helpful to do a demonstration of how to create an epidermal peel before learners start. Or you could re-play the appropriate part of the master video.) In pairs, learners collect a leaf from each plant and prepare their slides. They carry out their method for finding the stomatal density for each leaf. You should encourage more confident learners to find the stomatal density by using a graticule (so the unit would be 'number of stomata / mm^2'). You might need to support their use of the graticule and/or refer them to Worksheet B for a reminder of the required calculations. Less confident learners can find the stomatal density as 'number of stomata / field of view'. Make sure that each learner gets a turn to prepare a slide

Timings	Activity
	and use the microscope. Check that learners are recording their data as they collect it. **Safety** Circulate the classroom at all times during the experiment so that you can make sure that your learners are safe and that the data they are collecting is accurate.
	**Plenary** Ask learners to process their data in order to generate mean values.

Teacher notes

For the list of materials required to set up the atmosphere domes **six weeks** ahead of the *Lab lesson*, and for instructions of how to do this, please see Teacher Instructions 2.

For the experiment that learners will carry out, watch the *Teacher walkthrough* video and read the notes below.

Each group will require:

- a leaf from each of three plants (pre-incubated for six weeks in different CO_2 concentrations)
- graph paper
- 3 × white tiles
- 3 × watch glasses
- tweezers
- scalpel/sharp knife
- microscope
- 3 × microscope slides
- 3 × coverslips
- eosin stain in dropper bottle
- fine paintbrush
- permanent marker pen
- dropping pipette
- distilled water
- paper towel
- glycerol
- mounting needle
- timer

Safety

The information in the table below is a summary of the key points you should consider before undertaking this experiment with your learners. The information is **not** exhaustive and does not include storage or handling instructions.

Learners should always wear gloves, eye protection and lab coats. There should not be any eating or drinking in the lab. Hands should be washed thoroughly after handling eosin stain.

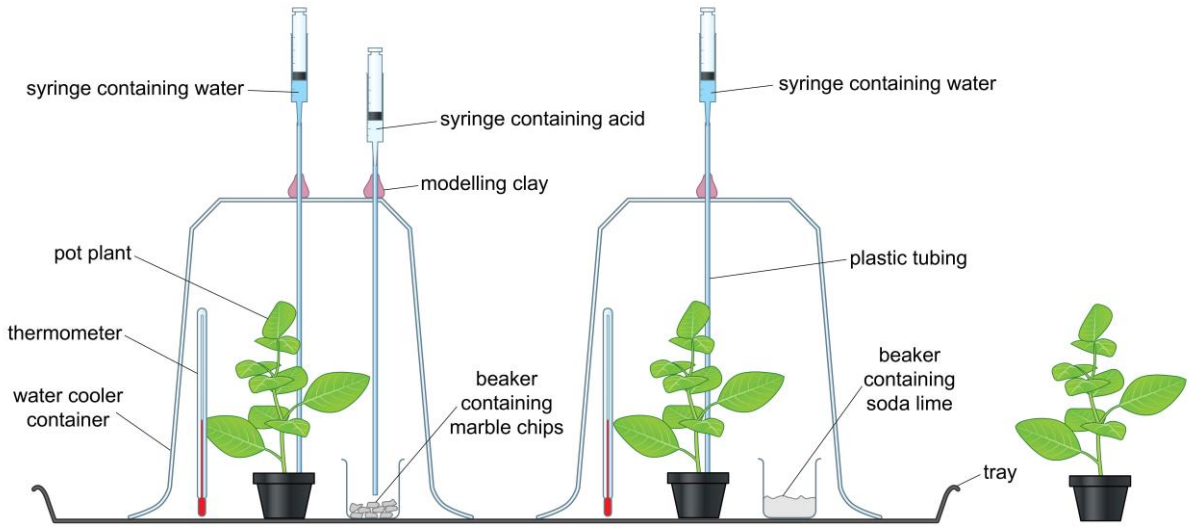
It is your responsibility to carry out an appropriate risk assessment for this experiment.

Substance	Hazard	First aid
Hydrochloric acid (dilute) [0.1 mol / dm^3] [0.5 mol / dm^3] [1.0 mol / dm^3]	 GHS07 (*moderate hazard* **MH**) [below a concentration of 2.7 mol / dm^3]	**In the eye:** Flood the eye with gently running tap water for 10 min. See a doctor. **Vapour breathed in:** Remove to fresh air. Call a doctor if breathing is difficult. **Swallowed:** Do no more than wash out the mouth with water. Do not induce vomiting. Sips of water may help cool the throat and help keep the airway open. See a doctor. **Spilt on the skin or clothing:** Remove contaminated clothing, then drench the skin with plenty of water. If a large area is affected or blistering occurs, see a doctor. **Spilt on the floor, bench, etc.:** For release of gas, consider the need to evacuate the lab and open all windows. For large spills, and especially for (moderately) concentrated acid, cover with mineral absorbent (e.g. cat litter) and scoop into a bucket. Neutralise with sodium carbonate. Rinse with plenty of water. Wipe up small amounts with a damp cloth and rinse it well.
Soda lime	 GHS05 (*corrosive* **C**)  GHS07 (*moderate hazard* **MH**)	**In the eye:** Flush the eye with gently running tap water for 20 min. Remove contact lenses if present and easy to do. See a doctor. If a visit to hospital is necessary, continue washing the eye during the journey in an ambulance. **Vapour breathed in:** Remove to fresh air. Call a doctor if breathing becomes difficult. Do not use mouth-to-mouth method if victim ingested or inhaled the substance; give artificial respiration with the aid of a pocket mask equipped with a one-way valve or other proper respiratory medical device. Administer oxygen if breathing is difficult. **Swallowed:** Wash out the mouth with water. Do not induce vomiting. Drink plenty of water. See a doctor. **Spilt on the skin or clothing:** Remove and isolate contaminated clothing and shoes. Wash the skin immediately with soap and rinse with plenty of water for at least 20 minutes (for minor skin contact, avoid spreading material on unaffected skin). See a doctor if irritation or symptoms persist. **Spilt on the floor, bench, etc.:** In the event of spillage, take up mechanically (e.g. sweep or vacuum up) into tightly closed containers. Adhere to personal protective measures. Flush any remainder with plenty of water. Label container and dispose of as prescribed **Note:** effects of exposure (inhalation, ingestion or skin contact) to substance may be delayed.

Substance	Hazard	First aid
Eosin	 GHS07 (*moderate hazard* **MH**)  GHS08 (*health hazard* **HH**)  GHS02 (*flammable* **F**)	**In the eye:** Flush the eye with copious amounts of gently running tap water for at least 15 min. Ensure adequate flushing by separating the eyelids with fingers. Remove contact lenses if present and easy to do. See a doctor. **Vapour breathed in:** Remove to fresh air. Call a doctor. If not breathing, give artificial respiration. **Swallowed:** Wash out the mouth with water. Do not induce vomiting. Loosen tight clothing such as a collar, tie, belt or waistband. Drink lots of water. See a doctor. **Spilt on the skin or clothing:** Remove and isolate contaminated clothing and shoes (these should be cleaned immediately or destroyed by burning). Immediately wash the skin with soap and flush with plenty of water for at least 15 minutes. Call a doctor. **Spilt on the floor, bench, etc.:** For small spills, add absorbent (soil may be used in the absence of other suitable materials), scoop up material and place in a sealed, liquid-proof and Clean-up Methods container for disposal. For large spills, block spilled material or otherwise contain material to ensure run-off does not reach a waterway. Place spilled material in an appropriate container for disposal. Minimise contact of spilled material with soils to prevent run-off to surface waterways.
Glycerol	Possible irritant	**In the eye:** Flush the eye with copious amounts of gently running tap water for at least 15 min. Ensure adequate flushing by separating the eyelids with fingers. Remove contact lenses if present and easy to do. See a doctor. **Vapour breathed in:** Remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Call a doctor. **Swallowed:** Wash out the mouth with water. Do not induce vomiting. See a doctor. **Spilt on the skin or clothing:** Remove and isolate contaminated clothing and shoes (these should be washed before use). Immediately wash the skin with soap and flush with plenty of water for at least 15 minutes. Call a doctor. **Spilt on the floor, bench, etc.:** Absorb spill with inert material (e.g. vermiculite, sand or earth), then place in suitable container. Avoid runoff into storm sewers and ditches which

Substance	Hazard	First aid
		lead to waterways. Clean up spills immediately, observing precautions in the Protective Equipment section. Remove all sources of ignition. Provide ventilation.
Cuts or puncture wounds due to sharps (scalpels, knives, dissection scissors, cork borers, mounted needles, broken glassware). Wounds can lead to infection, especially if the blade or point is contaminated.		**Minor cuts:** Rinse the wound with water. Get the casualty to apply a small, sterile dressing. **Severe cuts**: Lower the casualty to the floor. Raise the wound as high as possible. If feasible, ask the casualty to apply pressure on or as close to the cut as possible, using fingers, a pad of cloth or, better, a sterile dressing (adding further layers as necessary). If the casualty is unable to do so, apply pressure yourself, protecting your skin and clothes from contamination by blood if possible. Leave any embedded large bodies and press around them. Call a doctor.
Allergies – latex gloves		Remove the gloves and wash hands under water. Look out for severe allergic reactions such as difficulty breathing and/or swelling of the face, body or tongue. Seek emergency medical attention immediately.

Experiment set-up



Teacher method

This is your version of the method that accompanies the *Teacher walkthrough* video.

Before you begin

Plan how you will set up the plants to grow for six weeks in their respective atmospheres. One way of constructing suitable atmospheres is shown on Teacher Instructions 2, but other designs could be improvised. At the start of the six-week period, obtain some initial data (mean stomatal count per field of view for each plant and mean stomatal count per mm^2) to supply to your learners.

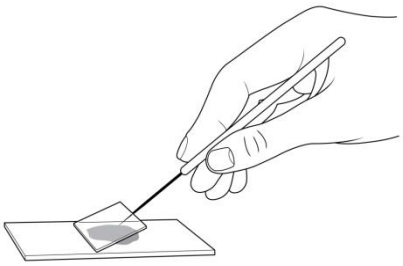
Think about:

- if learners will be involved in maintaining the plants during the six-week period or not
- if you have access to plants with a leaf epidermis suitable for making a peel
- the number of groups you will need for the final sampling (group size 2 learners)
- if your learners will use graticules or some other method to measure the field of view, or simply use 'field of view' as the unit of area.

Experiment

Walk around the learners during the experiment session in case they encounter any difficulties.

Steps	Notes
1. Learners should draw a table for all of their results and add the initial data for each leaf supplied by you.	*Learners should be encouraged to incorporate columns for anticipated data processing in their table at the outset; in this case for mean count, area of field of view and stomatal density as well as replicate stomatal counts.*
2. Learners should collect the apparatus they require from the front of the class.	
3. Each pair should pick one leaf from each plant.	*They should select a leaf from a similar position in each of the three plants, this is a way to ensure that the leaves are of similar age. For example, they could standardise the position by always picking the third leaf down from the apex.*
4. The leaves should be placed onto labelled white tiles.	*Tiles should be pre-labelled 'high CO_2', 'low CO_2' or 'atmospheric CO_2' using a permanent marker to avoid mixing up the leaves.*
5. Learners should draw around the outline of each leaf on graph paper and find the leaf area by counting squares.	*This confirms that the leaves are of a similar size and so are of similar age. If one of the leaves is much smaller/larger, a replacement leaf should be picked.*

Steps	Notes
6. Learners prepare an epidermal peel from each leaf by following Worksheet D.	*A quick demonstration may help learners to follow this procedure.*
7. They should then stain the epidermal peels with eosin by using either Option 1 or Option 2 from Worksheet D. (The instructions that follow assume Option 1 has been used.)	*It is important that the epidermal peels are not mixed up during the batch preparation; keeping them on their labelled white tiles will help avoid confusion.*
8. Slides should be labelled 'high CO_2', 'low CO_2' or 'atmospheric CO_2' using a permanent marker.	
9. Learners should transfer each peel to the correct microscope slide using a fine paint-brush or tweezers.	*Make sure that the learners ensure the peel is laid out flat; the fine paintbrush can be useful to unfold any overlapping parts.*
10. They should add a drop of glycerol on top of each peel, and then carefully place the coverslip over the top, avoiding trapping air bubbles.	*The coverslip should be placed at an angle over the sample, and then a mounting needle can be used to gently lower the coverslip.* 
11. Learners should view each epidermal peel under the microscope in turn; sampling their view and recording their stomatal counts per field of view in their table.	If using graticules, learners measure the radius of the field of view for the appropriate objective lens, calculate the area of the field of view used for each sample and therefore find the number of stomata / mm^2.
12. They calculate and record the final mean stomatal density for each leaf.	

Clean-up

After each experiment session learners should:

- tidy up their work space
- return all equipment to you.

Alternative methods

If you do not have access to the required equipment or the suggested method would not work for your class, here are some possible alternatives that you could use.

Epidermal impressions:
If your plants do not have leaves with an epidermis suitable for peeling, it is possible to make epidermal impressions using clear nail varnish. A small patch of clear nail varnish is painted onto the lower epidermis and allowed to dry. The nail varnish is then peeled off with fine forceps and mounted on a slide (or by placing a piece of sticky tape over it so that the impression sticks on the tape and can be transferred to the slide that way). However, this method takes longer than a direct epidermal peel because of the drying time required (10–15 mins), so your learners may find it hard to complete all three samples. Also, as no stain is involved, it can be difficult to see the stomata down the microscope.

Six-week incubation period:
If the apparatus and/or time for six weeks' exposure of the plants to altered carbon dioxide concentration is not readily available, you could amend the investigation such that learners compare the stomatal densities of leaves from two species of plants adapted to different conditions such as light and shade.

Debriefing lesson: Is the difference significant?

Resources	• completed data tables from the *Lab lesson* • Worksheets F and G
Learning objectives	By the end of the lesson: • ***all*** learners should be able to calculate the difference between the initial and the final stomatal density for each plant • ***most*** learners should be able to plot these differences on a bar graph with a negative section of the *y* axis • ***some*** learners will be able to interpret the results in terms of the outcome of a suitable statistical test.

Timings	Activity
10 min	**Starter/Introduction** Ask your learners to process their data by determining if there is a change in the mean number of stomata / field of view or number of stomata / mm^2 for each plant. They should present their processed data in a separate table. Explain that they need to find the difference between the initial and final density for each plant. The table should clearly indicate whether changes are increases or decreases, perhaps by using +/- symbols.
15 min	**Main lesson** Ask your learners to suggest how this data could be presented. They may need prompting to suggest a bar chart. Make sure they understand that a bar chart with separated bars is most appropriate because the data is categorical (three plants grown in three different conditions). Since the changes may be increases or decreases, they might need a negative section on their *y* axis so their bars can be drawn above or below the zero line. An example chart is given in Worksheet F if required. Learners draw a chart for their processed data.
25 min	Ask learners how they might determine if any of the differences are statistically significant. If they have already covered all four statistical tests in previous lessons they might be able to recognise, with prompting, that counted data is nominal rather than continuous so a chi-squared test is most appropriate. If your learners are not yet familiar with all the statistical tests, they could use Worksheet G to help them decide on the most appropriate one. If they know how to, learners carry out chi-squared tests on their data. A suggested way to carry out the chi-squared test is provided in the answers to Worksheet G. Ask learners to interpret their results in terms of the outcome of the statistical test. They should write a conclusion and explain the observed relationship. For example: '*Growing a plant for several weeks in higher than normal carbon dioxide concentrations results in a statistically significant decrease in the stomatal density of new leaves. This is because at higher than normal carbon dioxide concentrations the carbon dioxide diffusion gradient is steeper so sufficient carbon dioxide is able to diffuse into leaves through fewer stomata.*'

Timings	Activity
	**Plenary** Ask learners to suggest further investigations they could do on stomatal density. For example, ask them to suggest different species of plant they could compare, such as light- and shade-adapted species, and what they would expect to see.

Worksheets and answers

	Worksheet	Answers
For use in *Briefing lesson*:		
A: Under the microscope	**22**	**–**
B: Calculating stomatal density	**23**	**32**
C: Sampling stomata	**24**	**33**
Teacher instructions 1: Field of view	**29**	**–**
For use in *Planning lesson*:		
D: Planning to detect a difference	**25**	**34–35**
For use in *Lab lesson*:		
E: Setting up a light microscope	**26**	**–**
Teacher instructions 2: Setting up the atmospheres	**30–31**	**–**
For use in *Debriefing lesson*:		
F: Example chart	**27**	–
G: Which statistical test?	**28**	**36**

Worksheet A: Under the microscope

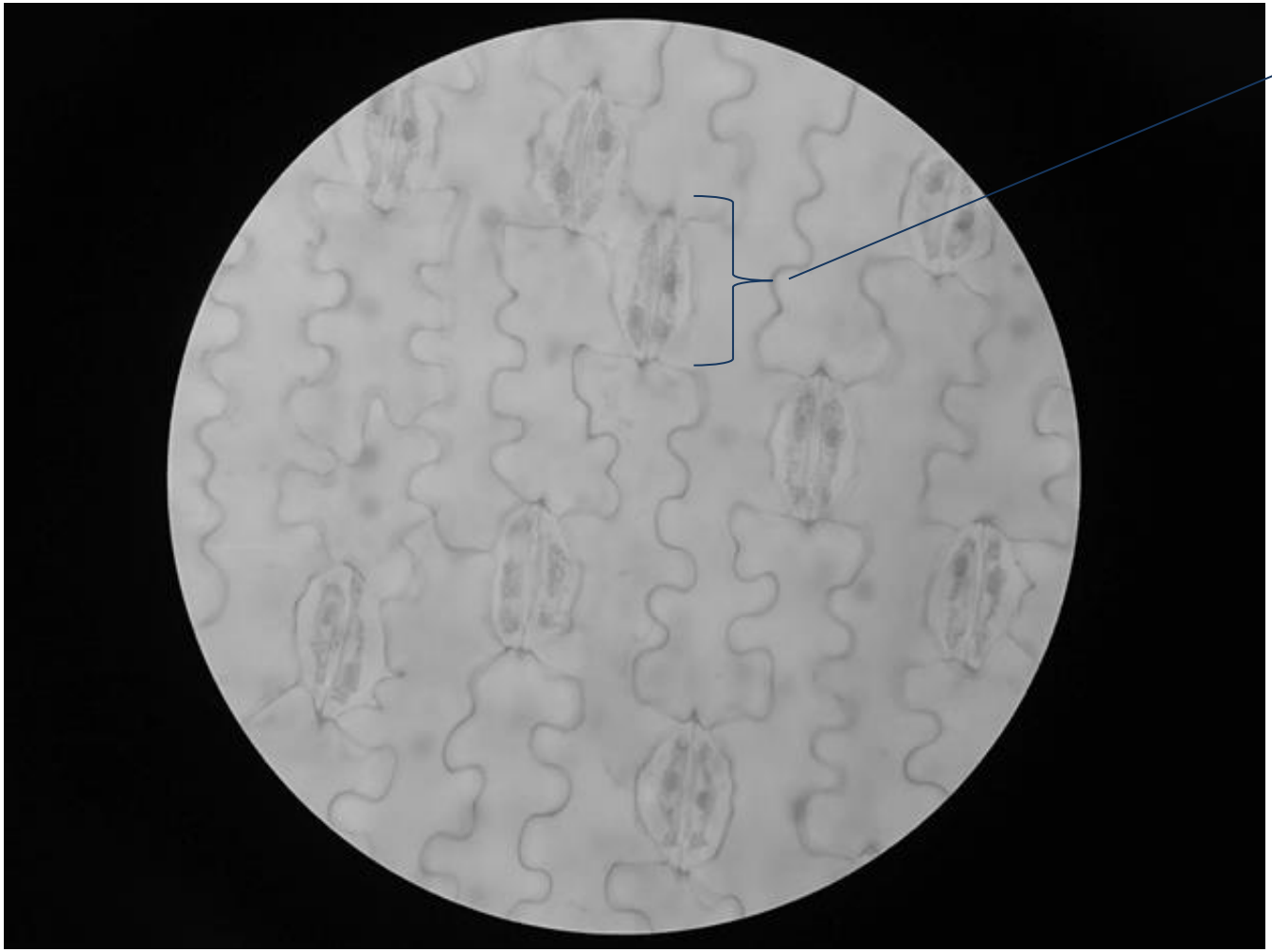


Figure 1: Lower leaf epidermis under a light microscope.

Worksheet B: Calculating stomatal density

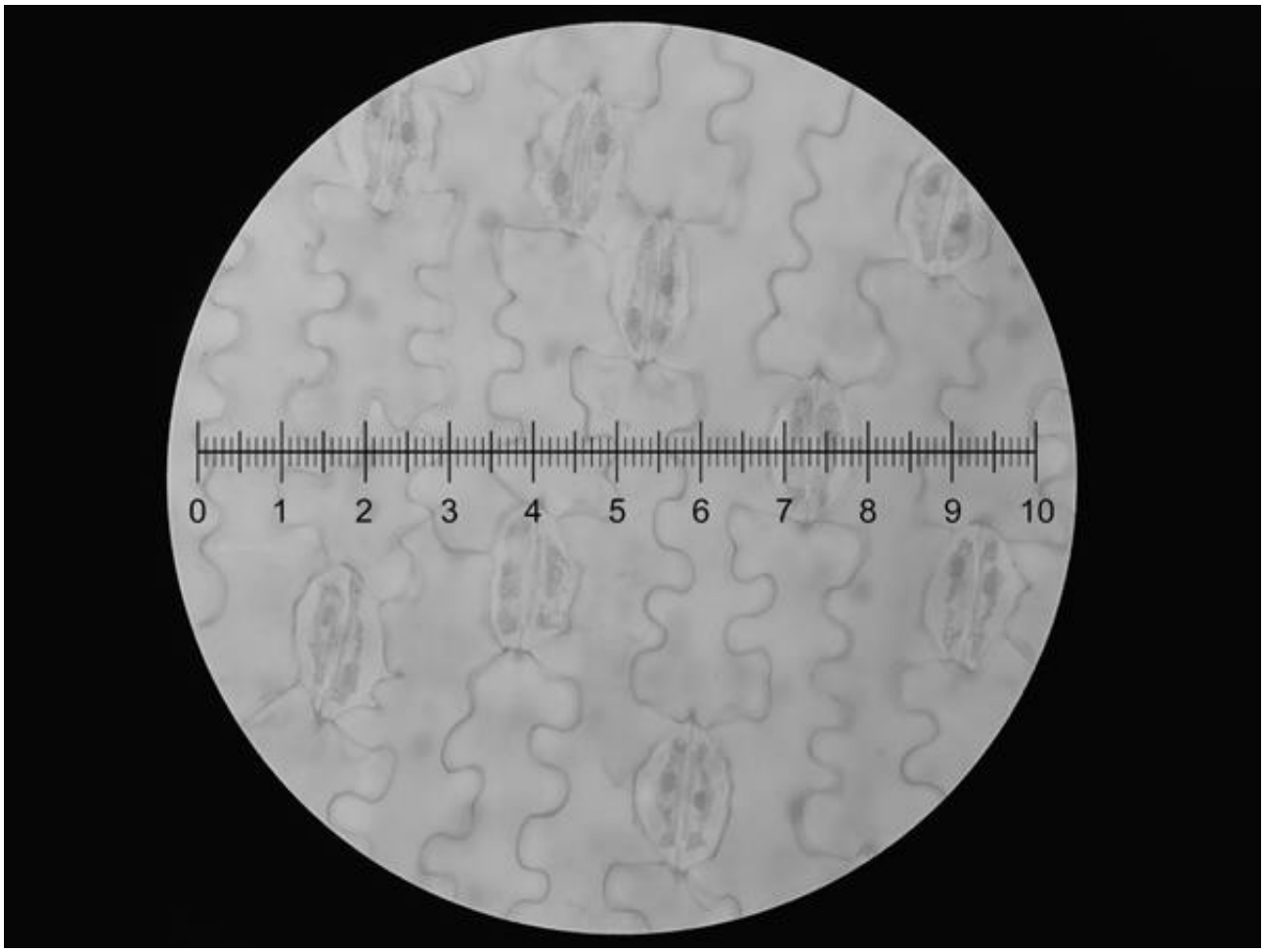


Figure 1: Lower leaf epidermis with graticule visible; 1 small graticule unit is equivalent to 3 μm.

Use the photo of a view down a light microscope to calculate the stomatal density of the sample

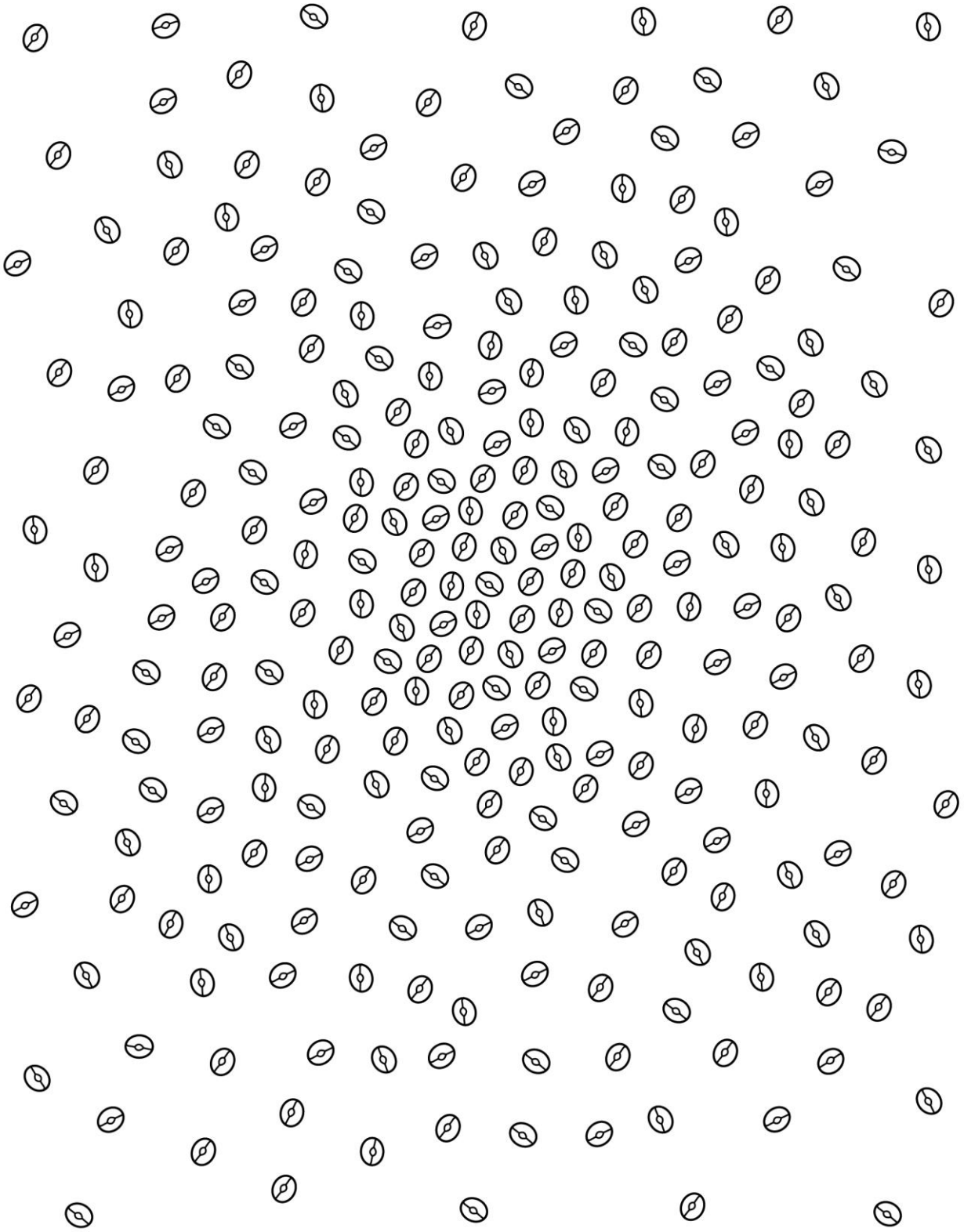
(a) in the field of view
(b) per square millimetre (show your working)

(a) Stomatal density = _____ stomata / field of view

(b)

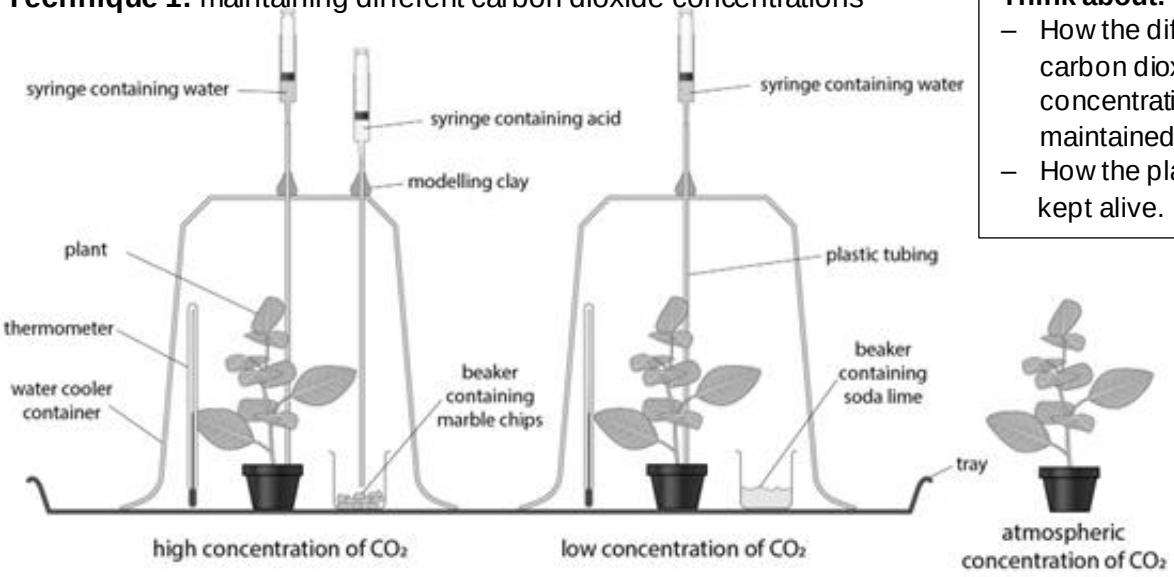
Stomatal density = _____ stomata / mm^2

Worksheet C: Sampling stomata



Worksheet D: Planning to detect a difference

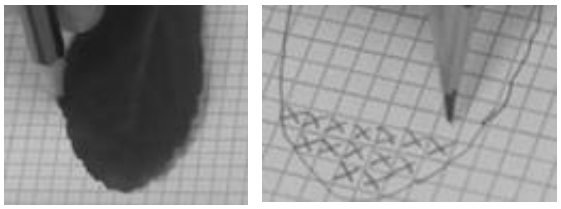
Technique 1: maintaining different carbon dioxide concentrations



Think about:
- How the different carbon dioxide concentrations are maintained.
- How the plants are kept alive.

Technique 2: Measuring the surface area of a leaf

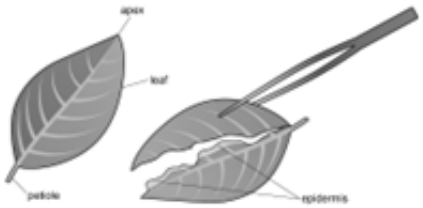
1. Place the leaf flat onto a piece of graph paper.
2. Draw around the leaf using a sharp pencil.
3. Count the number of squares.



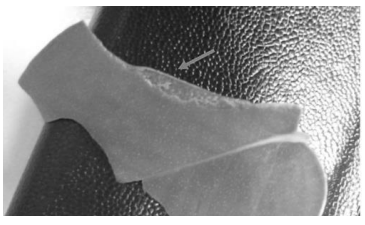
Think about:
- How to count partial squares.
- How to use this method to standardise the leaves you pick.

Technique 3: Cutting and staining an epidermal peel

1. Tear the leaf along a vein, from apex to petiole.
2. Identify the epidermis along the torn edge – it is a thin transparent layer.
3. Remove a piece of epidermis with an area of at least 3 mm^2.
4. To stain the peel you can use either Option 1 or Option 2.



Option 1:
- use a pair of tweezers to place the peel onto a watch glass
- add 2–3 drops of eosin stain and leave for 2 minutes
- move the peel to a watch glass containing distilled water to remove excess stain
- transfer it to a microscope slide, add a drop of glycerol and cover with a coverslip.

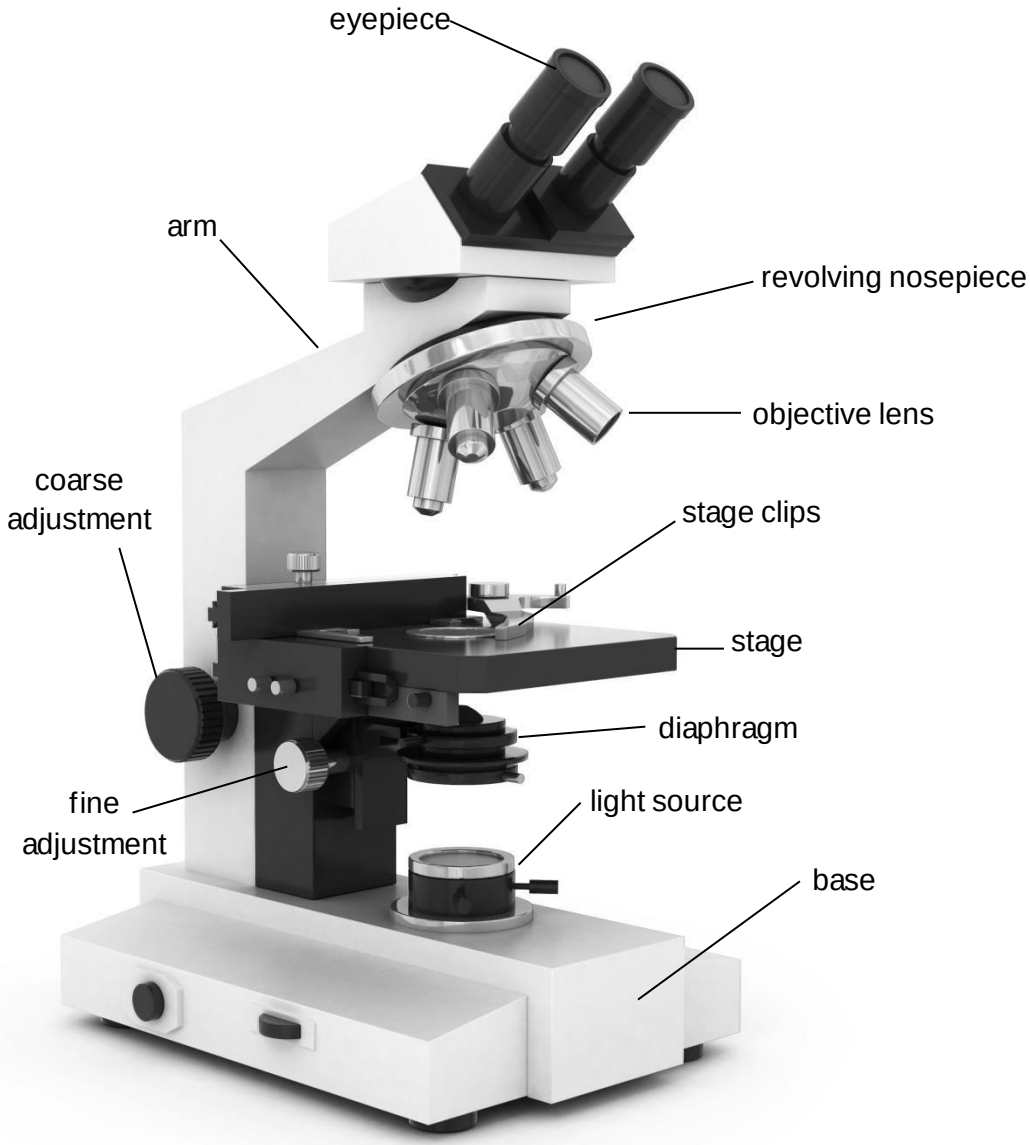


Option 2:
- use a pair of tweezers to place the peel onto a microscope slide
- add a drop of eosin stain, then place a coverslip over the top
- place a paper towel at the edges of the coverslip to soak up excess liquid.

Think about:
- Which side of the leaf the epidermis should come from.
- Why the eosin is added.
- Why the eosin is left for 2 minutes staining in Option 1.

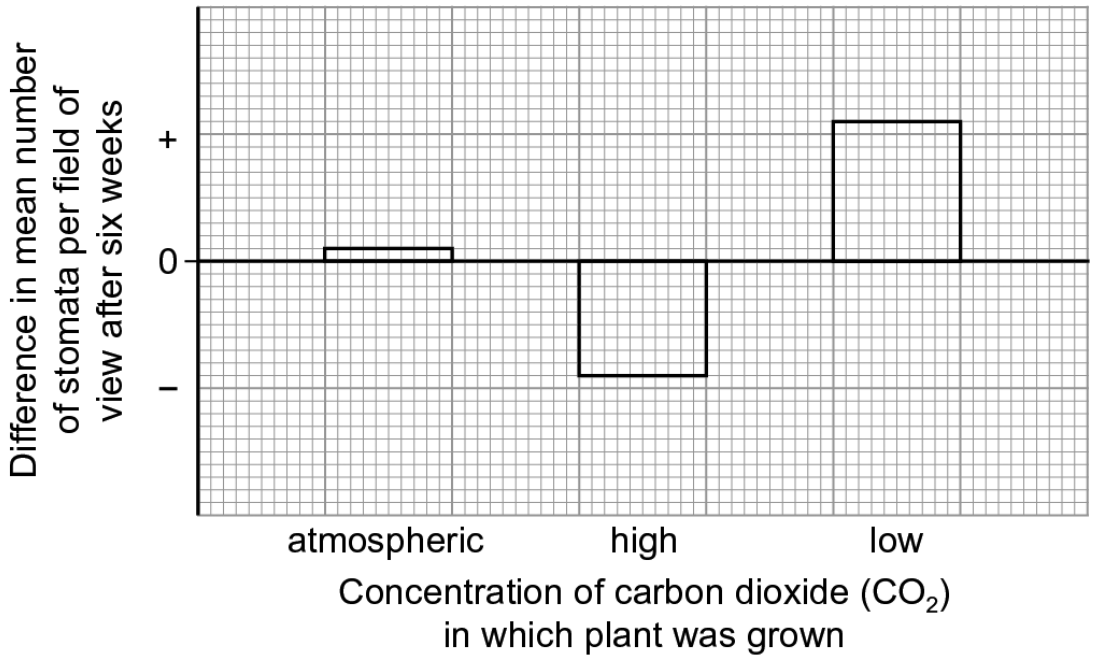
Worksheet E: Setting up the light microscope

1. Carry the microscope by holding the arm in one hand and supporting the base with the other hand.
2. Switch on the light source.
3. Open the diaphragm to its maximum aperture.
4. Place the slide on the stage and secure it under the stage clips with the epidermal peel positioned over the central hole.
5. Rotate the nosepiece so that the low power objective lens is in place.
6. Raise the stage as far as possible using the coarse adjustment; be careful not to hit the slide with the objective lens.
7. Look through the eyepiece.
8. Move the stage downwards with the coarse adjustment until the epidermal peel is in focus.
9. Rotate the nosepiece so that a higher power objective lens is in place.
10. Use the fine adjustment to adjust the focus until stomata are visible.



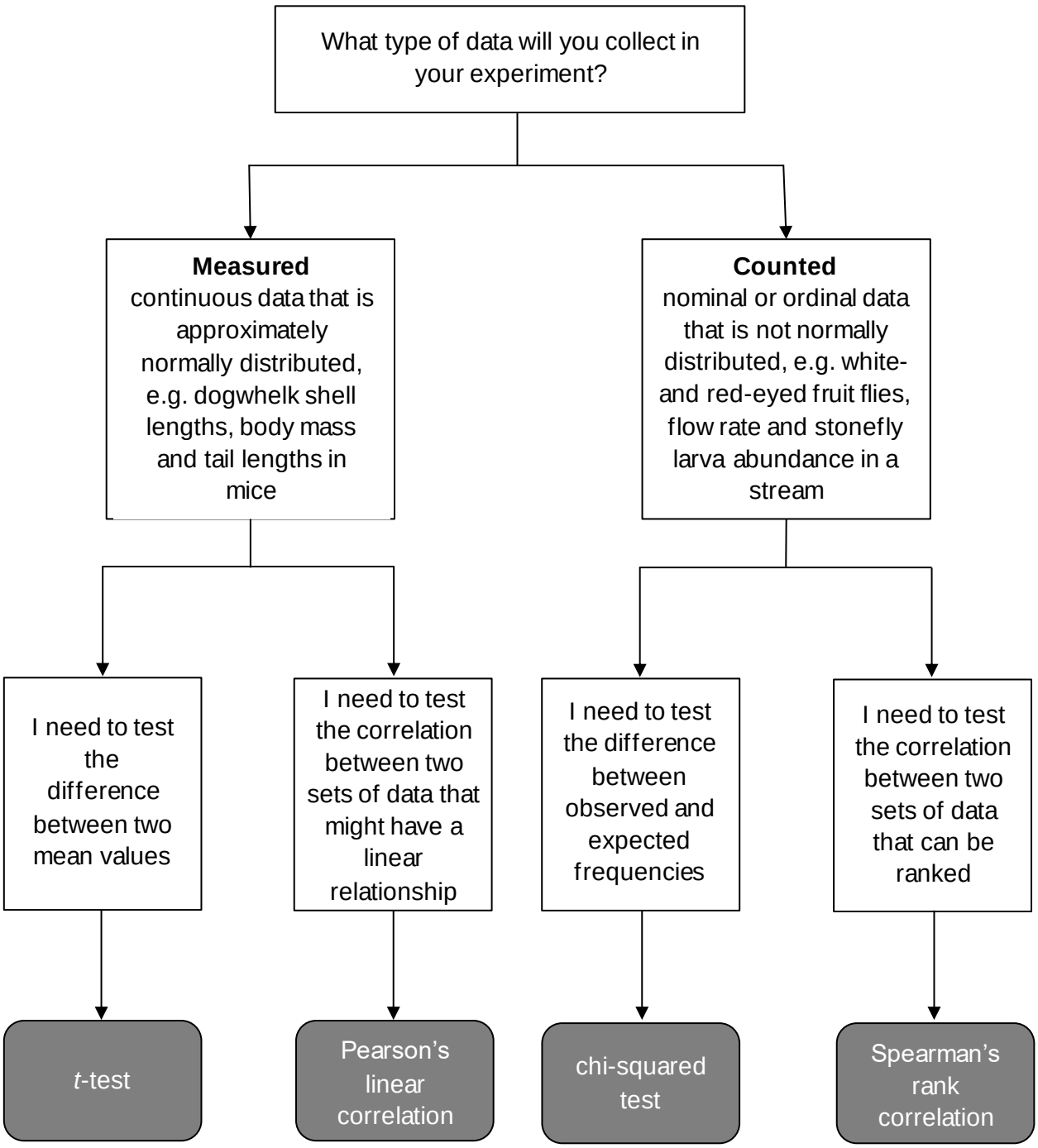
Worksheet F: Example chart

This is an example bar chart for the data collected using the method in the *Lab lesson*.



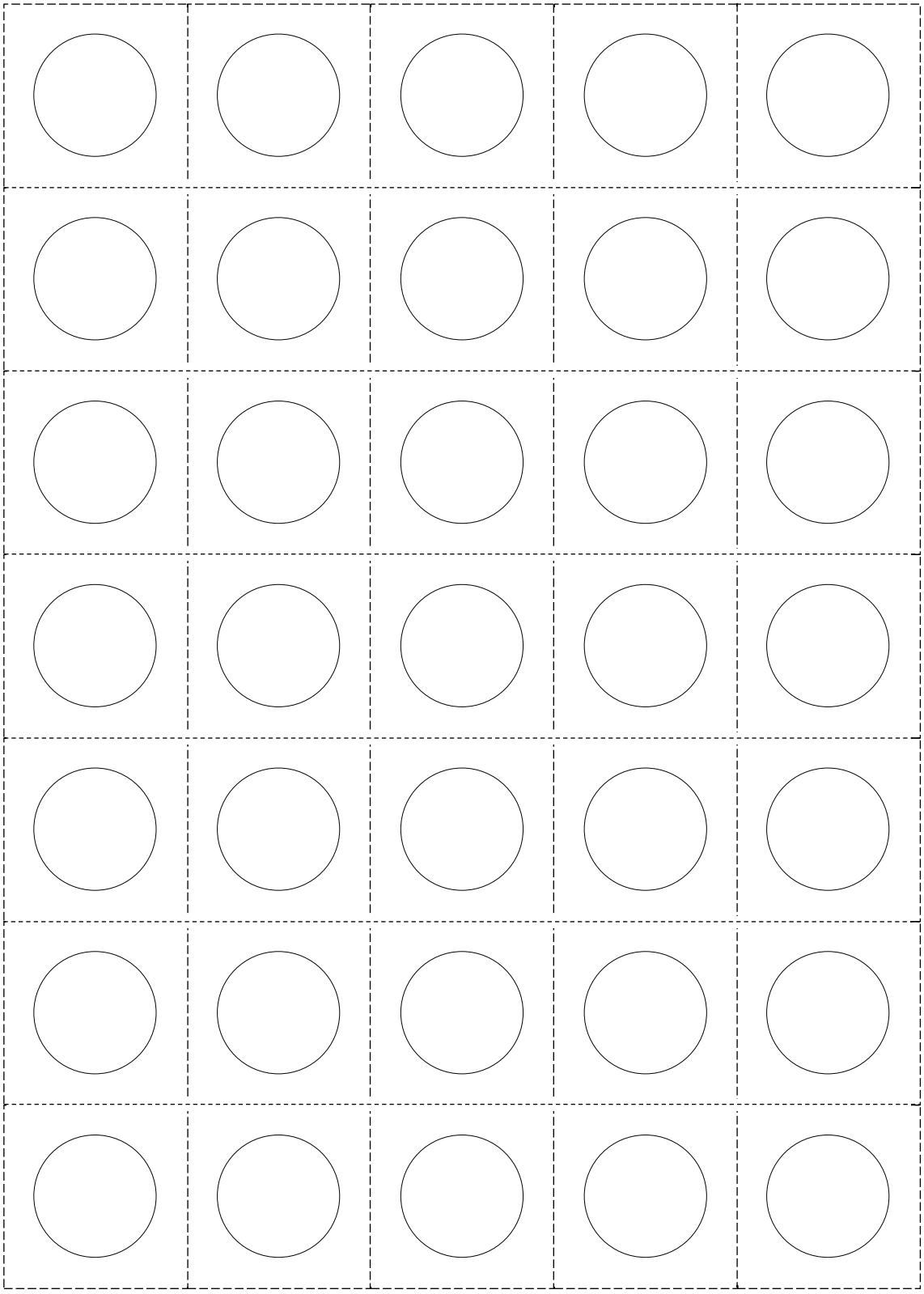
Worksheet G: Which statistical test?

Use the flow diagram to help you decide which statistical test is most appropriate for your data.



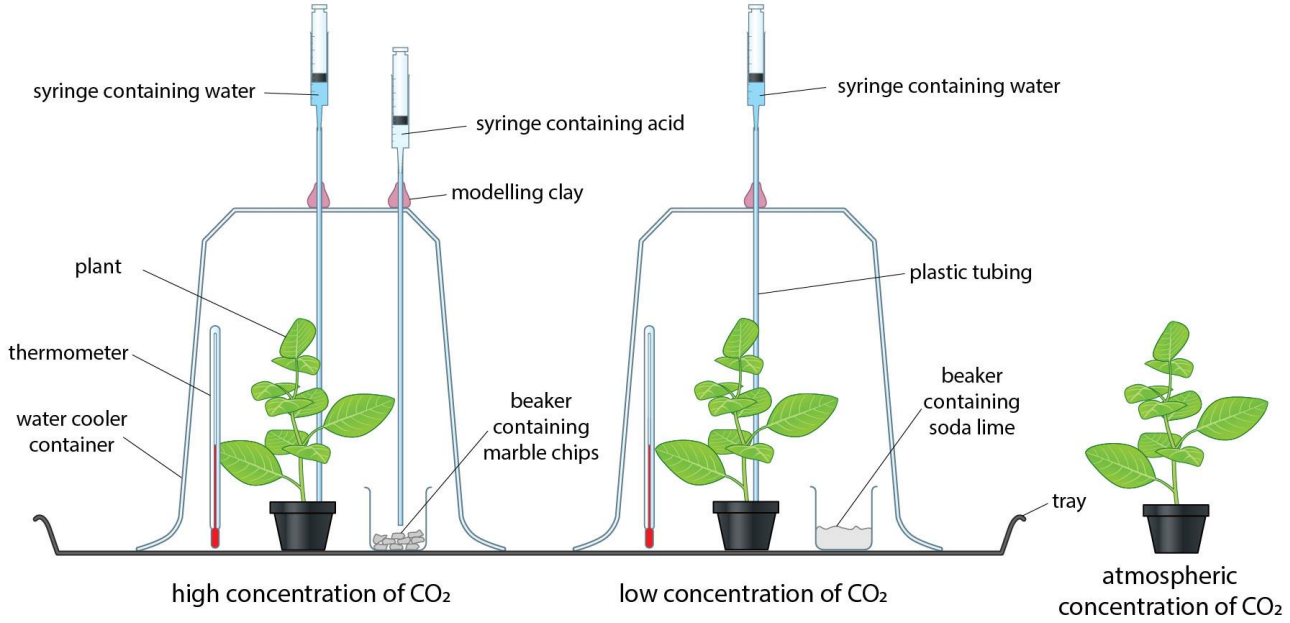
Teacher Instructions 1: Field of view

Photocopy this page onto clear acetate. Then cut along the dotted lines to provide each learner with one square each.



Teacher Instructions 2: Setting up the atmospheres

The different atmospheres can be created by making two sealed (air-tight) containers. The high carbon dioxide concentration is created and maintained by adding hydrochloric acid to marble chips; an atmosphere with low carbon dioxide concentration is made using soda lime.



Incubating the plants for six weeks will require:

- 3 × plants of approximately the same size – the plants should have leaves from which it is easy to peel off the epidermis, e.g., basil (*Ocimum*); geranium (*Pelargonium*); 4 o'clock plant (*Mirabilis*); red hot poker plant (*Kniphofia*); elephant-ear saxifrage (*Bergenia*); spider plants (*Chlorophytum comosum*); and others.
- modelling clay (a sphere of 2–3 cm)
- plastic tubing (total length around 1 m, to fit the two syringe nozzles)
- 2 × 250 cm^3 beakers
- 2–3 spatulas of large marble chips (calcium carbonate)
- 0.5M hydrochloric acid (100 cm^3 total)
- 5 g soda lime
- 4 × watch glasses
- 2 × thermometers
- 2 × small saucers
- 25 cm^3 syringe
- 10 cm^3 syringe
- sealant or strong glue (to make an air-tight seal)
- 2 × transparent domes, e.g. a plastic bell dome; plastic garden bell cloche; or large plastic water cooler container with funnel end removed
- 2 × plastic trays (large enough to fit the transparent domes)
- Equipment required to carry out an epidermal peel and to calculate the stomatal density of three leaves, one leaf from each plant, see Teacher Method.

Instructions

Make sure you collect data for the mean stomatal density for each plant **before** you set up the experiment as per the instructions below. Your learners will need this data, as they will only be finding stomatal density **after** the six-week period. You should provide data for **both** 'number of stomata / field of view' and the 'number of stomata / mm^2' so that you have the appropriate data for whichever method your learners use (unless graticules are not available and so only the 'field of view' will be used and not 'mm^2').

If you are using water cooler containers, remove the top of the containers (the part with the funnel) using a saw; this should leave a container that will stand on a flat surface.

1. Label one incubator 'High CO_2' and drill **two** holes into the base (which is the 'roof' once the container is upturned) – these must fit the plastic tubing securely; one hole in the centre and one towards the side.
2. Label the other incubator 'Low CO_2' and drill **one** hole into the base (which is the 'roof' once the container is upturned) – this must fit the plastic tubing securely; the hole should be in the centre.
3. Tape a thermometer to the inside of each container; make sure the scale is facing outwards so that the thermometer can be read from outside the container, through the wall of the container.
4. Prepare a piece of plastic tubing for each drilled hole; it should be long enough that it sticks out of the top of the container by 10 cm and touches the base inside the container; place each piece of tubing into the respective holes.
5. Add 2–3 heaped spatulas of large marble chips to a 250 cm^3 beaker.
6. Place the beaker of chips onto a tray.
7. Place one of the plants on a saucer, and the saucer onto the tray.
8. Place the 'High CO_2' container over the top of the tray, positioning the rubber tubing so that the central one drops into the plant's pot, and the tubing off to the side drops into the beaker of marble chips.
9. Use modelling clay to seal the holes around the tubing in the roof of the container.
10. Use a strong glue/sealant to glue the container to the tray; make sure all points are sealed (air-tight) around the base and the holes in the roof.
11. Add 5g of soda lime into a 250 cm^3 beaker.
12. Place the beaker of soda lime onto the other tray.
13. Place one of the plants on a saucer, and the saucer onto the tray.
14. Place the 'Low CO_2' container over the top of the tray, positioning the plastic tubing so it drops into the plant's pot. (Nothing needs to be added to the soda lime so there is no need for this to have a tube.)
15. Use modelling clay to seal the hole around the tubing in the roof of the container.
16. Use a strong glue or sealant to glue the container to the tray; make sure all points are sealed (air-tight) around the base and the hole in the roof.
17. Fit syringes containing water or acid to the plastic tubes as shown in the diagram.

Every **2–3 days** use the syringe to add 25 cm^3 of tap water to each plant via the appropriate plastic tubing. Also every **2–3 days**, use the syringe to add 10 cm^3 of hydrochloric acid to the marble chips in the same way. If new leaves are not growing, you might need to adjust the frequency of watering. Make sure the plants are placed in an appropriate amount of light, and are all kept in the same place so they are at the same temperature.

Worksheet B: Answers

(a) 9 stomata / field of view

(b) The diameter of the field of view is 109 small graticule units (by extending graticule to edges of the field of view (FOV)).

Each small graticule unit is equivalent to 3 μm, so diameter of field of view is 109 × 3 = 327 μm

The radius (r) is therefore $\frac{327}{2}$ = 163.5 μm

The area of the field of view = πr^2

$= \pi \times 163.5^2$

$= 83981\ \mu m^2$ (1 sf)

$= 0.084\ mm^2$

There are 9 stomata clearly visible in the field of view.

So, there is 9 stomata in 0.084 mm^2 but the unit is per 1 mm^2, so

$\frac{1}{0.084} \times 9 = 107$

So the stomatal density is 107 stomata / mm^2

Worksheet C: Suggested methods

Your learners should use a grid or coordinate method for randomising the placing of their samples. They should not simply put the acetate circle onto the worksheet.

Method 1:
One suitable method would be to place rulers on two sides of the worksheet and generate random pairs of numbers that can be used as coordinates. So for example, (2, 6) would mean that the centre of the acetate circle would be placed at the intersection of lines extending from 2 cm on one ruler and 6 cm on the other ruler.

Method 2:
Another method would be to draw a grid onto Worksheet C. The grid would need to be of small squares, not larger than 2 cm × 2 cm. The gridlines could be numbered or lettered and again, random pairs of numbers or letter combinations generated.

In both cases, pairs of numbers can be generated by rolling dice or by picking folded pieces of numbered paper from a beaker. The key point is that human bias in positioning the samples is avoided.

Running means:
A running mean for the number of stomata counted in each sample will indicate when the number of samples is sufficient enough to be representative of the whole area. When the running mean hardly changes with further samples, then the sample is representative. The table shows an example where seven samples reaches a representative value.

Sample	Number of stomata in sample	Mean number of stomata per sample
1	8	N/A
2	11	9.5
3	9	9.3
4	8	9.0
5	10	9.2
6	8	9.0
7	9	9.0

Worksheet D: Example outline plan

The example below is a model plan as written from the perspective of a learner.

The effect of carbon dioxide concentration on the stomatal density of growing plants

The independent variable will be the concentration of carbon dioxide the plant is grown in. This will be changed by keeping the plants in sealed containers with controlled atmospheres. The dependent variable will be the stomatal density of young leaves at the end of the growing period. The stomatal density will be found by looking at pieces of leaf epidermis from the younger leaves using a microscope.

It is not possible to set up a range of carbon dioxide concentrations. Instead, two carbon dioxide concentrations will be used. An atmosphere with a higher than normal carbon dioxide concentration will be created by adding acid to marble chips in a transparent sealed container. An atmosphere with a lower than normal carbon dioxide concentration will be created by placing soda lime in a transparent sealed container. A plant will be placed inside each container and allowed to grow for six weeks. The plants will get light through the transparent containers but will need to be watered via a syringe and plastic tubing because the containers must stay sealed.

A control will be set up by using a third plant which will be kept beside the two containers in normal atmospheric air. This will show that any change in stomatal density in the other plants is due to the different carbon dioxide concentrations.

The light intensity, temperature and amount of moisture in the plant pots will need to be kept the same for all three plants because these factors may also have an effect on the stomatal density of growing leaves. This will be done by keeping all three plants in the same place and watering each plant with the same volumes of water. The leaf epidermis from each plant must be taken from leaves of the same age. To check that leaves are the same age, their areas will be measured by drawing around the leaves on graph paper and counting squares. The leaves used to find stomatal density will be picked to have very similar areas and any that do not will be replaced.

To see if the stomatal density of young leaves has changed during the six weeks, the stomatal densities will need to be found at the start, before the growing period, and again at the end of the six weeks. The young leaf chosen from each plant will be torn in half and a small piece of lower epidermis will be pulled off. The lower epidermis has more stomata. This will be put onto a microscope slide with a little eosin stain. The epidermis is transparent so the stain makes the stomata easier to see.

Random fields of view will be chosen by moving the slide to different positions against scales made from strips of graph paper stuck to the microscope stage. Random pairs of numbers from rolling dice will be used to move the corner of the slide up and across by so many squares. The number of stomata in each field of view will be counted and this will be repeated until the mean number per field of view becomes constant. This value will be recorded for a young leaf taken from each plant at the start and again at the end of the six weeks so any change can be seen. A statistical test could be used to see if any change is statistically significant.

The experiment is mainly low risk. Hydrochloric acid and soda lime are more hazardous and should be handled carefully, but will be inside an enclosed container for most of the time.

Worksheet G: Example chi-squared test

Nominal data such as stomatal counts are not normally distributed, so finding the significance of any difference in stomatal density after the six weeks should be done using the chi-squared test. Assuming that carbon dioxide concentration has no effect on stomatal density, the frequency of stomata found on the young leaves of each plant should be the same at the beginning and the end of the growing period. A significant difference would indicate that carbon dioxide concentration does have an effect.

Below is an example chi-squared test using example data.

	High CO_2 concentration	Low CO_2 concentration	Atmospheric CO_2 concentration
Initial mean number of stomata / field of view	62	59	65
Final mean number of stomata / field of view	40	63	67

Given the frequencies of the stomata recorded initially, and with no effect of carbon dioxide concentrations on stomatal density, you would expect the proportion of the total number of stomata that is present in each condition to be the same before (initial) and after (final) incubation. For example,

62 + 59 + 65 = 186 stomata in total

There were 62 stomata in the 'high' sample, which as a proportion of the total number is $\frac{62}{186} = 0.33...$, which means that 33% of the total stomata (186) were found in the high sample. So, you would expect the same proportion of stomata to be in the high sample after incubation:

40 + 63 + 67 = 170 So, calculate 0.33… × 170 = 56.7 (1 dp)

So the expected and observed frequencies after incubation are:

	High CO_2 concentration	Low CO_2 concentration	Atmospheric CO_2 concentration
Observed final number of stomata / field of view	40	63	67
Expected final number of stomata / field of view (1 dp)	56.7	53.9	59.4

The chi-squared value is calculated as follows $\chi^2 = \sum \frac{(O-E)^2}{E}$

	High CO_2 concentration	Low CO_2 concentration	Atmospheric CO_2 concentration
$(O–E)^2$ (2 dp)	278.89	82.81	57.76
$(O–E)^2/E$ (2 dp)	4.91	1.54	0.97

$$\chi^2 = \sum \frac{(O-E)^2}{E} = 7.42$$

Since the chi-squared value of 7.42 is above the critical value of 5.99 for $p = 0.05$ and 2 degrees of freedom, the results show a significant difference in stomatal density due to different carbon dioxide concentrations.

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