Cambridge Assessment International Education

Teaching Pack

Investigating mitosis by preparing a root tip squash

Cambridge International AS & A Level Biology 9700

Version 1.0

Cambridge Pathway

Contents

Icons used in this pack:

Briefing lesson

Planning lesson

Lab lesson

Debriefing lesson

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.

↓

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

↓

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 mitosis by preparing a root tip squash

This *Teaching Pack* focuses on the preparation of a root tip squash for viewing with a light microscope, in order to identify cells in different stages of mitosis.

This practical activity enables learners to visualise the four key stages in mitosis, as seen in meristem cells from tissue in the root tip. It can be used as a basis of an investigation to determine the effect of a named variable on the mitotic activity of dividing cells.

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

- 5.2. Chromosome behaviour in mitosis

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:

- 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
- observing cells under the light microscope
- drawing cells as seen under the light microscope to include only the observable features
- drawing the distribution of different tissues in a plant specimen and labelling
- estimating the number of cells in a field of view using a sample
- (optional) calibrating an eyepiece graticule using a stage micrometer
- Obtain actual sizes of cells using a calibrated eyepiece graticule, by measuring the cells to obtain a mean measurement, using the correct units for cell microscopy (μm).

Prior knowledge

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

- 1.1. The microscope in cell studies
- 1.2. Cells as the basic units of living organisms
- 5.1. Replication and division of nuclei and cells

Briefing lesson: The stages in mitosis

Resources	• Worksheets A and B • Photo of each stage of mitosis (see Teacher Instructions 1) • Teacher instructions 1 and 2 • 4 × A4 size envelopes • 7 sticky labels (10 cm length) • Rulers (30 cm) • A4 paper (1 per group of learners) • Calculators • Alarm clock and/or bell that is audible to all learners.
Learning objectives	By the end of the lesson: • ***all*** learners should be able to identify the four key stages of mitosis from the behaviour of chromosomes in cells • ***most*** learners should be able to describe the events that occur in the four key stages of mitosis • ***some*** learners should be able to explain the importance of the four stages of mitosis and how they relate to cell position and size of cells in the root tip of plants.

Timings	Activity
10 min	**Starter/Introduction** Before the start of the lesson, follow Teacher Instructions 1 and print out seven images, ideally on A3-sized paper, showing cells undergoing different stages of mitosis. Ask seven volunteers to hold up one of the seven images each, so that they can all be seen by the whole class. Ask the rest of the class to rearrange the volunteers in the correct order to show the process of mitosis from start to finish. At the end of the activity, make it obvious that the learner who is first in the line should link up with the learner who is last to highlight that the process of mitosis is a cycle, punctuated by interphase. After the activity concludes, you could fix the images to the classroom wall in the correct order so that they are clearly visible, to serve as a visual aid for some of the upcoming tasks. Write the names of the stages in the space provided on the photographs for learners' reference.
40 min	**Main lesson** Learners will carry out a series of four tasks in groups at different 'stations' (locations) in the classroom. Before the start of the lesson, make sure that you have prepared the 'stations' according to the instructions on Teacher Instructions 2. You should place the appropriate page of Worksheet A at each station. You can either provide enough copies for each learner, or just one per group. Inform learners that they will take part in a 'skills circuit' that consists of four collaboration tasks that they must complete in groups (2–6 learners per group, depending on the class size). Explain that these will develop their skills in key areas that are required to communicate effectively in biology. Explain that they will only have 10 minutes to carry out each task and must therefore work together quickly. When the alarm sounds they must stop what they are doing and move on to the next task, which

Timings	Activity
	will be at a different table/station. Point out where they should sit in order to undertake the four tasks, then divide learners into their groups, and tell each group which station they will start at. Each task relates to skills they will need in the *Lab lesson.* Circulate between the groups during the activities to support and stretch learners' thinking. After each 10-minute period, ring a bell or have an alarm set, or otherwise inform learners that they have to move on to the next station. Once all groups have completed the activity at each station, summarise the most important learning outcomes in a class discussion. Emphasise that these tasks highlight a number of very common misunderstandings and misconceptions when communicating data and conclusions. These include the fact that descriptions and explanations of scientific data are often interchanged (Tasks 1 and 2); the incorrect division of factors by incorrectly using the magnification equation (Task 3); and using shading or sketchy lines in scientific diagrams to add depth or light and dark (Task 4). Highlight that the skills that the learners have developed in this activity are of great importance in order to communicate accurately in biology.
	**Plenary** Give each learner Worksheet B. They should take a few minutes to think about the answers, reflecting on the tasks of the main lesson to help them. Then they should be asked to discuss the answers with a partner for another 2 minutes, before writing down their final answers. If necessary, prompt discussion to elicit any missing content knowledge about where in plants mitosis is most easily observed (root tips and shoot tips); and that meristem tissue consists of roughly circular or square-shaped undifferentiated cells with a nucleus that is large relative to their overall size. All learners then circulate around the room to compare and contrast their final answers. Ask learners to think about how they might improve their answers now that they have discussed them with others. This should reinforce both the content knowledge required for the subsequent lessons, and put into practice some of the communication skills developed during the main lesson.

Planning lesson: A moment in time

Resources	• Worksheets C and D • '*Investigating mitosis by preparing a root tip squash*' video • Learner access to the internet and/or a course textbook • Optional – garlic clove with sprouting roots suspended above a beaker of water using a toothpick, see Teacher notes
Learning objectives	By the end of the lesson: • ***all*** learners should be able to outline how to prepare a root tip squash to visualise meristematic cells • ***most*** learners should be able to describe how and when key reagents and procedures are used in the preparation of a root tip squash • ***some*** learners should be able to describe why key reagents and procedures are used in the preparation of a root tip squash.

Timings	Activity
15 min	**Starter/Introduction** Give pairs of learners Worksheet C. Ask them to look at **Figure 1** and discuss how long a person undertaking a 3-hour visit to the fairground might spend doing six activities: paying to enter; choosing a ride; queuing up to get on a ride; sitting on a ride; queuing up to buy food; and eating food. Ask learners to work with their partner to discuss and describe an analogy with the stages of mitosis that are shown in **Figure 2**. By asking for contributions in a whole-class discussion, you should elicit that some activities take longer (e.g. queuing up to sit on a ride; prophase) than others (e.g. sitting on a ride; anaphase). Therefore, if a photograph was taken at any given moment in time, then it's likely that more visitors, or cells, will be caught carrying out a given activity if that activity takes longer compared to others. Hence, the probability of finding a cell in a certain stage of mitosis is correlated with the length of time that a cell remains in that stage. Elicit from learners that it is important to count the total number of cells in the field of view (or the total number of people at the fairground), so that a *proportion* of cells at a given stage of mitosis can be calculated. This allows for comparison of different root tip samples, which may represent different days at the fairground. More able learners could be challenged to describe how such an image could be used to estimate the duration of a given phase, e.g. the time spent by a cell in metaphase, if they are given the total duration of the cell's cell cycle. This can be done by using the following formula, which relies on knowing the approximate length of a cell cycle in the garlic root. The duration of a cell cycle in garlic meristematic cells is around 24 hours: $\text{Estimated time of a cell cycle phase} = \left(\frac{\text{number of cells in this phase}}{\text{total number of cells}}\right) \times \text{total length of cell cycle}$
25 min	**Main lesson** Explain to learners what is meant by a 'root tip squash'. Ask why you might use a root tip squash, rather than a preparation of tissue from a different region of the plant, to observe cells underdoing mitosis under a light microscope. Learners should realise from the previous lesson that plant root tips contain meristem tissue in which the

Timings	Activity
	undifferentiated cells are undergoing mitosis. Inform them that in the next lesson, they will carry out a root tip squash of their own. Give each learner Worksheet D and explain that it contains a series of problems that they need to solve if they are to produce a successful root tip squash and view cells down a microscope. The solution to each 'problem' is a specific step that forms part of the method. Tell them that in the *Lab lesson* they will be provided with a clove of garlic which has grown fresh roots. (Explain that it was 'set to root' over a beaker of water for 2–3 days; you might wish to demonstrate this and show a garlic clove with sprouting roots; see Teacher notes for how to grow the garlic roots.) Encourage learners to work individually for 20–25 minutes, making use of internet research, their textbook and/or other resources to help them solve the 'problems' associated with preparing a slide for a root tip squash. The worksheet challenges the learners to consider *why* key reagents and procedures are necessary in the preparation of a root tip squash, rather than simply listing *what* is needed.
	For 5–10 minutes, ask the learners to discuss their findings with two other peers in mixed-ability groups. They should consider how the findings of their research agrees with and differs from those of others, but they should **not** yet make any changes to their decisions. A whole-class discussion could follow if time allows.
	**Plenary** Play the demonstration video and challenge learners to modify and develop their answers on Worksheet D. The task is intended to ensure that all learners have a clear idea of the procedure and the underlying basis of each step, prior to undertaking the experiment themselves in the next lesson. The completed worksheet should be read and reviewed for homework, in advance of the *Lab lesson*, or it could be collected by you for formative assessment and to ensure that the class has a common understanding of the task ahead. Inform learners that they will begin the experiment immediately at the beginning of the next lesson.

Lab lesson: Getting practical

Resources	• Worksheets E, F, G, H and I • All equipment as per *Teacher notes*
Learning objectives	By the end of the lesson: • ***all*** learners should be able to follow instructions to successfully prepare and identify root tip meristem cells under a microscope • ***most*** learners should be able to successfully prepare, identify and record diagrams of root tip meristem cells in different stages • ***some*** learners will be able to suggest how aspects of the method used to prepare and identify root tip meristem cells could be improved.

Timings	Activity
45 min	**Main lesson** Learners should have their completed Worksheet D for reference, to help remind them why each step in the method is required. Give learners Worksheet E, which contains the step-by-step instructions they should follow and Worksheet G, on which they are asked to draw cells. If learners are inexperienced or lack confidence with using a light microscope, give them Worksheet F. More able learners could be asked to calculate the actual size of viewed cells using an eyepiece graticule and stage micrometer; Worksheet H provides support for developing this skill. As they undertake the experiment, ask learners to identify any steps in the method with which they experienced problems or difficulties; these notes will be useful when they come to evaluate the procedure in the *Debriefing lesson*. **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.
15 min	**Plenary** Learners will be at different stages of the practical activity towards the end of the lesson, with some likely to need the full hour to completely finish. An extension activity for learners who finish first is provided on Worksheet I; this will help to develop their thinking with regard to the experiment. This requires them to demonstrate some of the skills they developed in the *Briefing lesson*, namely description, explanation, drawing diagrams and calculating magnification. You may wish instead to provide this to learners at the beginning of the lesson, to promote independent thinking during periods of waiting time during the practical and also during the packing away of equipment. It is important that all learners have completed Worksheet I in advance of the *Debriefing lesson*, as they will need to know about the concept of the mitotic index for the activities ahead. You may wish to set the worksheet as homework for learners who do not have time to complete it at the end of this lesson.

Teacher notes

For the *Lab lesson*, the learners should be provided with garlic cloves that already have roots.

You need to leave garlic roots to grow for at least 3 days before the *Lab lesson* is carried out.

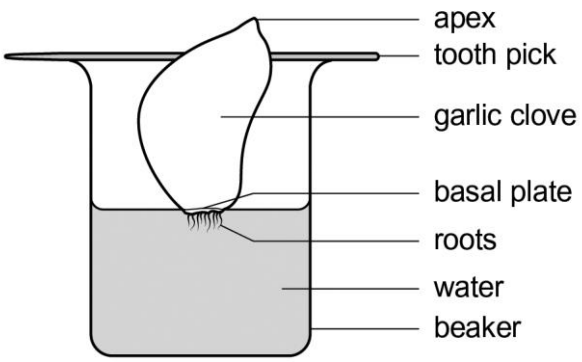
You will need:

- garlic gloves (one per learner, this allows for 'extras' in case roots do not grow)
- toothpicks
- 50 cm^3 beaker (or similar container)
- tap water

Set up the apparatus as shown in the diagram below.

1. Pierce the apex of the garlic clove with a toothpick and push the garlic clove to the centre. (The toothpick should be inserted close to the apex, as this is far from the basal plate where the roots will grow).
2. Balance the toothpick and garlic clove on a 50 cm^3 beaker containing about 25 cm^3 of tap water.
3. Add / remove water using a dropping pipette until the basal plate of the garlic clove is just touching the surface of the water. (It is important that there is enough water in the beaker to completely immerse the basal plate, taking into account the fact that evaporation will occur during the three-day growing period.)
4. Leave for three days.

It is not necessary to top up the beaker with water each day (providing you don't leave it anywhere which encourages lots of evaporation). Leaving the set-up in the dark may encourage germination.



For the experiment that learners will carry out in the *Lab lesson*, watch the *Teacher walkthrough* video and read the notes that follow.

Each group will require:

- microscope (× 10 to × 40 magnification)
- 25 cm^3 of 1M hydrochloric acid
- dissection (sharp) scissors
- scalpel
- watch glass
- stopwatch
- 2 × 50 cm^3 beakers
- 2 × dropping pipettes
- 1–2 cm^3 glycerol
- white tile
- microscope slide
- 3 × coverslips
- fine tweezers or fine paintbrush
- paper towels
- garlic clove (*Allium* sp.) with sprouting roots, around 3 days old
- 1% toluidine blue stain (in dropper bottle)
- water bath set at 60°C
- hot plate set at 40°C.

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 at the end of the experiment.

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

Substance	Hazard	First aid
1 % toluidine blue stain	 GHS07 (*moderate hazard* **MH**)	**In the eye:** Check for and remove contract lenses. Flood the eye with gently running tap water for at least 15 min, hold the eyelids open. 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:** Do no more than wash out the mouth with water. Do not induce vomiting. Rinse the mouth thoroughly. Loosen tight clothing such as a collar, tie, belt or waistband. See a doctor. If large quantities of this material are swallowed, call a physician immediately. **Spilt on the skin or clothing:** Remove all contaminated clothes and footwear immediately unless stuck to skin. Wash the skin with plenty of soap and water. Cover the irritated skin with an emollient. Get medical attention if irritation develops. Cold water may be used. **Spilt on the floor, bench, etc.:** Dilute with water and mop up, or absorb with an inert dry material and place in an appropriate waste disposal container. Finish cleaning by spreading water on the contaminated surface and dispose of according to local and regional authority requirements. Large Spill: Absorb with an inert material and put the spilled material in an appropriate waste disposal. Finish cleaning by spreading water on the contaminated surface and allow to evacuate through the sanitary system.
Hydrochloric acid (dilute) [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.
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 (wash before reuse). 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 lead to waterways. Clean up spills immediately, observing precautions in the Protective Equipment section. Remove all sources of ignition. Provide ventilation.

Substance	Hazard	First aid
Food (generic)	ALLERGENS	Do not consume any foodstuffs in the labs. Gloves should be used if learners have allergies. If discomfort persists, see a doctor.
Latex gloves	ALLERGENS	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.

Hazard	First aid
Burns (hot water)	Flood burnt area with water for at least 10 minutes. For serious injuries, see a doctor.

Teacher method

This is the method that accompanies the *Teacher walkthrough* video.

Before you begin

Plan how you will group your learners during the experiment session.

Think about:

- when to carry out the lesson – the cell cycle is influenced by the plant's circadian clock, so active cell division is most likely to be observed in the morning or around midday, which is when the experiment should ideally be carried out
- the number of groups you will need (group size 2–3 learners)
- the amount of equipment/chemicals required
- the questions you could pose to learners during less active periods in the practical task, for example during incubation windows or during the clean-up.

Experiment

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

Steps	Notes
1. Learners should collect the equipment they require from the front of the class.	*Asking different learners to collect different items can improve the smooth running of the practical task.*
2. Learners should find a space in the classroom where the equipment can be assembled safely.	*Learners should be instructed that they should remain standing for this practical task.*
3. Make sure your learners are briefed with regard to aspects of safety, including the hazard warning symbols associated with the chemicals used in this investigation.	*The teacher or technical staff should prepare the toluidine blue stain and hydrochloric acid in advance of the lesson, and ensure they have clearly displayed hazard warning symbols.*
4. Learners should add 25 cm^3 of 1M hydrochloric acid to a 50 cm^3 beaker. Then place this into a water bath set at 60°C, for 10 minutes.	*The acid must be incubated for at least 10 minutes to bring it to this temperature.*
5. Whilst they are waiting for the acid to reach 60°C, learners should rinse their garlic gently but thoroughly with distilled water from a wash bottle.	*The toothpick should be left inside the clove for the whole experiment as this makes the handling of the glove in subsequent steps much easier. Washing at this point will remove any material that may have attached to the root tips during the growth period.*

Steps	Notes
6. Once the acid is at the correct temperature, learners should balance their garlic glove over the beaker of acid using the toothpick (the roots must be sitting in the acid). They should leave it in the water bath for 10 minutes.	*The hot hydrochloric acid breaks down the pectin lamellae between cell walls. This allows the stain to better penetrate the tissue in the next stage, and also ensures that it will be possible later to squash the tissue to a thickness of one cell.*
7. Learners should rinse their garlic glove gently but thoroughly with distilled water from a wash bottle.	*Any residue of hydrochloric acid remaining on the roots could limit the effectiveness of the stain.*
8. The clove is then held over a white tile and sharp scissors are used to cut off around half a centimetre of **three** separate roots.	*Using several roots increases the likelihood that one root tip will give a successful squash.*
9. A scalpel is then used to cut the very end 2–3 mm of tips from these cuttings. The rest of the material is discarded.	*Extreme care should be taken when using the scalpel; draw learners' attention to the fact that it is extremely sharp.*
10. Learners use fine tweezers to gently transfer the root tips to a watch glass. All three root tips should be placed within an area of approximately 1 cm^2 but they should not touch. This is to ensure that the stain will fully contact all parts of the tissue.	*A paintbrush may be used as an alternative, which has the advantage of limiting damage to the tissue. Any overlap between the root tips could minimise the extent to which the stain penetrates the tissue.*
11. Using the strain dropper, learners should add 1 drop of toluidine blue stain carefully to the watch glass to cover all three root tips.	*A stain is required in order to bind to the chromosomes and make them visible under an optical microscope.Toluidine blue is used to stain the DNA instead of the more traditional acetic orcein stain because it:* • *produces a better level of contrast within the cells* • *is less hazardous to learner health* • *less expensive than alternative stains.*
12. The watch glass is then placed onto a hot plate set at 40°C for 10 minutes. This period of incubation at a warm temperature encourages the stain to fully penetrate into the tissue.	*Leaners should **not** allow the stain to completely evaporate during the incubation period – they should remove the watch glass from the hot plate earlier if necessary, or add a drop of distilled water.*

Steps	Notes
13. After 10 minutes, the watch glass is removed from the hot plate and another drop of stain is added to the 3 root tips. This time however, the stain is immediately removed and replaced by a few drops of distilled water.	*The effectiveness of the staining procedure is increased by applying fresh stain and repeated washing with distilled water.*
14. This water is again removed, and replaced by a few more drops of fresh distilled water, which is also again removed. By this point, there should be no more obvious stain left in the watch glass.	*Any traces of the stain in the tissue that remain unbound to DNA may obscure clear viewing of the chromosomes.*
15. Fine tweezers are used to transfer the root trimmings to a single microscope slide.	*A paintbrush may be used as an alternative which has the advantage of limiting damage to the tissue.*
16. The root tips should be positioned around 2.5 cm apart from each other.	*Allowing for this distance between the tips will provide space for the three separate coverslips in the next stage.*
17. A small drop of glycerol is then put onto each root tip to keep them hydrated.	*Learners should avoid excess glycerol as pieces of tissue will drift to the edge of the coverslip and be lost in the next step. Excess glycerol should be removed using a paper towel placed at the edge of the coverslip.*
18. A coverslip is placed carefully onto each root tip, and care is taken to avoid introducing any air bubbles.	*A mounted needle can be used here to gently lower the coverslip onto the slide, which minimises the chance of air bubbles being trapped. Air bubbles would be artefacts that obscure the view of chromosomes.*
19. With care, the slide and specimens are then placed onto the middle of a folded paper towel, which is just slightly wider than the slide itself.	*Delaying squashing for several hours can allow the cells to harden, which reduces the chance of them bursting upon squashing.*
20. The paper towel is then carefully folded over the slide, so that both sides of the slide are covered. With care to avoid moving the cover-slips on top of the slide, the paper towel is carefully rolled up to completely wrap the paper towel around the slide.	*By wrapping the slide in many layers of material, there is less chance of the glass fracturing during the subsequent squashing step.*

Steps	Notes
21. A clenched fist is then used to apply continuous downward pressure to the slide. There should be no twisting or other sideways movements during this step, as this could damage the glass or the plant tissue.	*Ensure that the surface on which the 'squash' is performed is completely flat. This squashing has the effect of compressing the root tips in order to generate single layers of cells.*
22. The slide is then carefully removed from the paper towels, and is placed on the stage of a light microscope. The slide is viewed using the lowest magnification to locate cells. The magnification is increased to identify, more clearly, the meristem cells organised in a single layer.	*The meritstem cells are small and square-shaped and have a large nucleus. If cells are overlapping, obscuring a clear view of chromosomes, learners should wrap the slide and squash again: if one layer of cells is positioned on top of another, the view of the chromosomes will be obscured. If learners have introduced too many air bubbles, instruct them to add water to the corner of the coverslip using a fine dropping pipette); this should push the air bubbles out.*
23. Once some cells have been found, the highest objective lens is used to further magnify the field of view in this region. A magnification of at least × 400 is required to see chromosomes in the nucleus.	*Patient observation may reveal cells that are undergoing all four stages of mitosis.*

Clean-up

After the experiment learners should:

- bring any leftover chemicals to you
- clean all glassware
- tidy up their work space
- ensure any spillages have been mopped up.

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.

Alternatives to garlic cloves include: onions, hyacinths or seedlings of broad beans or peas.

Debriefing lesson: Critiquing

Resources	• Worksheet J • Glue • A3 paper (three sheets per group) • Sticky notes (10 per learner)
Learning objectives	By the end of the lesson: • ***all*** learners should be able to recognise that the technique of preparing a root tip squash could be used as a basis to investigate the effect of a named variable on the rate of mitosis in meristematic cells • ***most*** learners should be able to plan an investigation into the effect of a named variable on the rate of mitosis in meristematic cells • ***some*** learners should be able to suggest improvements to an investigation into the effect of a named variable on the rate of mitosis in meristematic cells.

Timings	Activity
10 min	**Starter/Introduction** To refresh their knowledge and understanding of the concepts they encountered in the previous lessons, arrange learners in pairs to run a revision activity. Inform them that they need to identify the 'odd one out'. They are given three terms and they need to discuss which one is less related to the other two terms. They must justify their decisions. Follow up with a whole-class discussion about the answers. Provide the terms so that the whole class can see them. Expected responses are shown in square brackets. 1. *anaphase*, *metaphase*, *prophase* [*anaphase*; because it is only in this stage of those listed in which chromosomes consist of separated sister chromatids] 2. *staining*, *squashing*, *heating* [*squashing*; because this is the only step of those listed in which the root tip is on the microscope slide] 3. *describe*, *explain*, *calculate*. [*explain*; because it is the only command term of those listed that requires the use of the underlying scientific concepts to justify a statement]. Note that the listed way in which each word is the 'odd one out' is not exhaustive, and can lead to some very insightful discussions and debates.
35 min	**Main lesson** Provide each learner with Worksheet J, which contains plans written by three students. The title of the investigation is included on the appropriate page of the Worksheet. After reading through the work, ask your learners to work in groups of 3–4 to critique each report and suggest improvements. Tell them to stick each report in the middle of a piece of A3 paper and annotate it with their comments of what is wrong and how it can be improved; they do this by writing labels on the surrounding A3 paper. Key mistakes (and hence areas for learners to suggest improvements) are provided in the answers section, in the answers for Worksheet J. Circulate to provide opportunities for support and stretch; make sure learners know that this activity mirrors the real-world process of scientific peer review.

Timings	Activity
	**Plenary** Learners need to use the knowledge and skills that they have encountered in the previous activity to agree on the most effective way of planning an investigation. Divide the board up into a number of different sections. Ask them to identify the key stages in a scientific investigation (e.g., hypothesis; apparatus (and materials); step-by-step method; data collection; data presentation; data analysis). Write each as a header to one of the sections you've created on the board. Ask learners to write down what they think (individually) is the most important consideration for each stage of an investigation. They should write this down on a sticky note for each stage, and then place it on the board in the appropriate section. The considerations should be general to all investigations. Develop a class discussion by looking at the sticky notes and asking who agrees/disagrees, and discuss alternative suggestions either by looking at more sticky notes or based on learners' verbal suggestions. The display could be kept for the next lesson to review learners' submissions further, or you could ask learners to use it to record the most appropriate suggestions and improvements, as identified by you. Learners could be encouraged to take photographs of the collective display, to make a digital record for future reference and revision.

Worksheets and answers

	Worksheet	Answers
For use in *Briefing lesson*:		
A: Skills circuit	**22–25**	**49–50**
B: More mitosis	**26**	**51**
Teacher Instructions 1: The order of stages in mitosis	**39–46**	**–**
Teacher Instructions 2: Circuit support	**47–48**	**–**
For use in *Planning lesson*:		
C: One moment in time	**27**	**–**
D: Planning a method	**28**	**52**
For use in *Lab lesson*:		
E: The method	**29–31**	**–**
F: How to use a light microscope	**32**	**–**
G: Observing cells	**33**	**53**
H: Eyepiece graticules and stage micrometers	**34**	**–**
I: An investigation involving mitosis	**35**	**54**
For use in *Debriefing lesson*:		
J: Student plan	**36–38**	**55–56**

Worksheet A: Skills circuit – Task 1

This task will develop your skills in **quantitative description**. Do **not** open the envelope until you have read the instructions below. The envelope contains a graph that relates to the process of cell division. Choose **one** member of the team to be the 'describer' – this should be someone who has not yet been a describer.

Instructions for the describer:

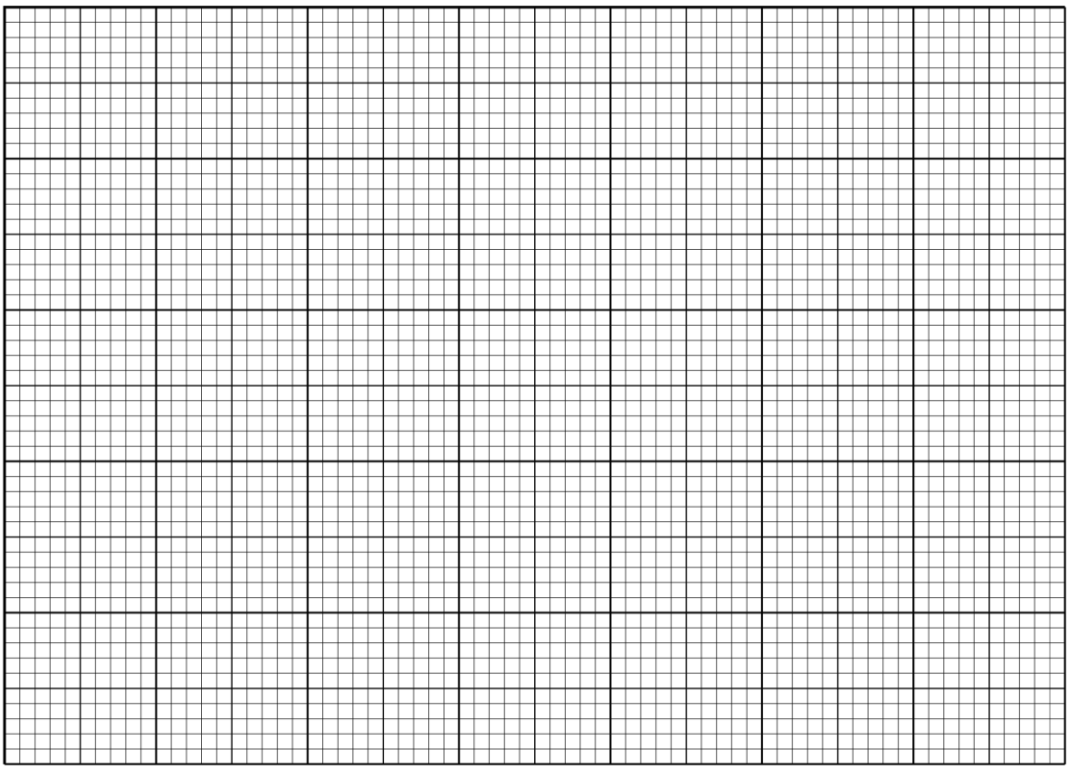
1. Open the envelope; do **not** show the rest of your group the image inside.
2. **Describe** the shape of the graph to the rest of your group. You must **not** draw anything on paper or use hand movements. You can only provide a **verbal** description.

Instructions for the rest of the group:

1. You must work as a team to agree on how to sketch what is being described.
2. Pick one member of the group to do the drawing; use the graph paper below.

Instructions for the whole group:

1. When you have all finished, the describer can show you the graph from the envelope. You should **all** compare your group's graph with the image. How is the shape of your graph similar or different to the image?
2. Discuss in your group how the description might have been different and why this might have allowed you to reproduce the graph more accurately.
3. If you finish this task before the 10 minutes are over, you should discuss the graph in your group and try to **explain** the shape of the graph to your teacher.



Worksheet A: Skills circuit – Task 2

This task will develop your skills in **qualitative description**. Do **not** open the envelope until you have read the instructions below.

The envelope contains a piece of paper showing an image of a cell as viewed down a light microscope. Choose **one** member of the team to be the 'describer'– this should be someone who has not yet been a describer.

Instructions for the describer:

1. Open the envelope; do **not** show the rest of your group the image inside.
2. **Describe** the image to the rest of your group. You must **not** draw anything on paper or use hand movements. You can only provide a **verbal** description.

Instructions for the rest of the group:

1. You must work as a team to agree on how to draw what is being described.
2. Pick one member of the group to do the drawing; use the space below.

Instructions for the whole group:

1. When you have all finished, the describer can show you the image from the envelope. You should **all** compare your drawing with the image. How is it similar or different to the image?
2. Discuss in your group how the description might have been different and why this might have allowed you to reproduce the diagram more accurately.
3. If you finish this task before the 10 minutes are over, you should discuss the image in your group and try to **explain** what it shows to your teacher.

Worksheet A: Skills circuit – Task 3

This task will develop your skills in **calculating magnifications** and the **actual sizes** of specimens. Work on your own to complete the task, then compare your work with the rest of the group when you have finished.

You should use the seven diagrams of the cells provided by your teacher to answer each question. You will also need a ruler and a calculator.

1. Calculate the magnification of **two** of the images provided.

Cell 1 – Use the space below to show your working.

Magnification: _____

Cell 2 – Use the space below to show your working.

Magnification: _____

2. Calculate the **actual size** of the cells in two of the images provided. Your answer should be given in micrometres (μm).

Cell 3 – Use the space below to show your working.

Actual size: _____ **μm**

Cell 4 – Use the space below to show your working.

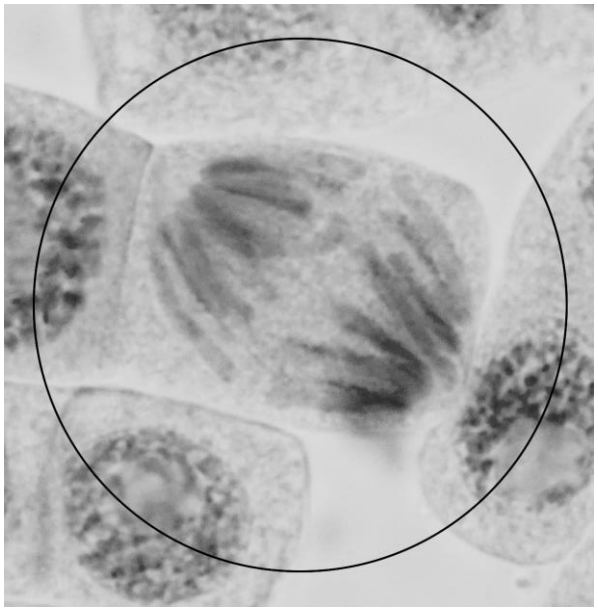
Actual size: _____ **μm**

When you have finished, compare and discuss your answers with other members of your group.

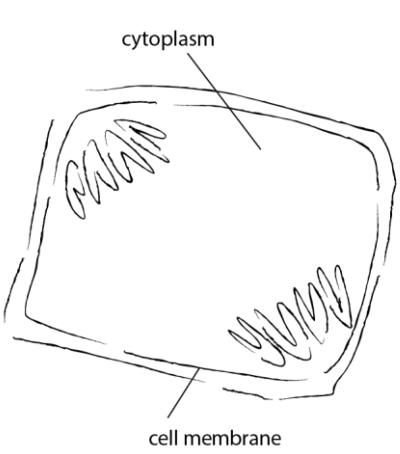
Worksheet A: Skills circus – Task 4

This task will develop your skills in **constructing a scientific diagram**.

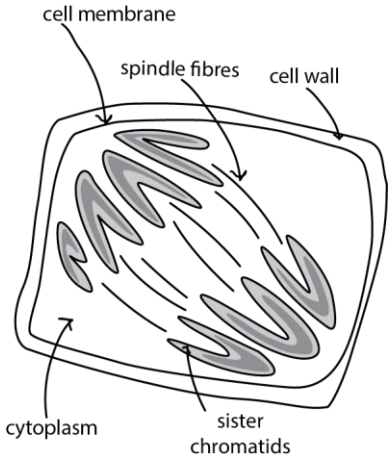
The photo shows some plant cells as seen under a light microscope. The circle shows the field of view. Three students were asked to draw a diagram of **one** cell as seen in the field of view. The cell is undergoing anaphase.



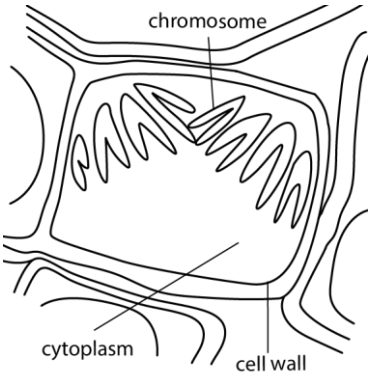
1. Discuss the three student drawings below and list, for each diagram, 2–3 reasons why they are not acceptable scientific diagrams.



Student 1



Student 2



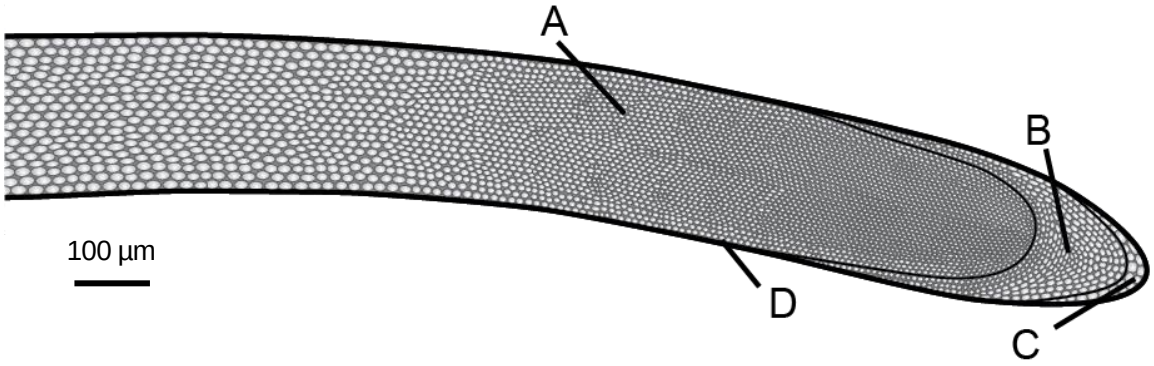
Student 3

2. Draw a labelled diagram of the cell in the photo above, using the correct techniques. Try not **to** repeat the mistakes shown in the three student diagrams.

Worksheet B: More mitosis

The diagram below shows a cross section of a root tip from the garlic plant.

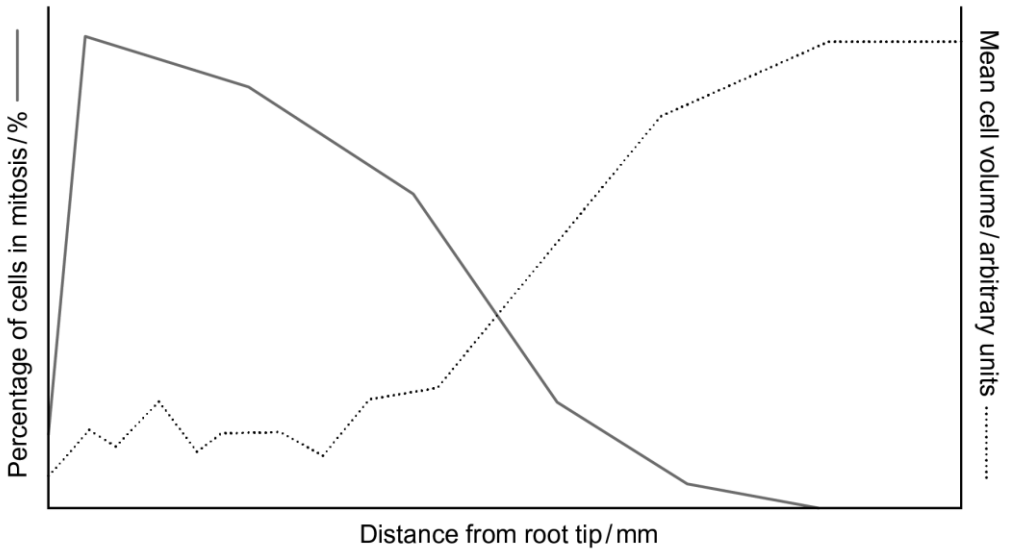
1. Identify in which region of the root tip most cells will be undergoing mitosis.
   Explain your answer.



2. Use the scale bar to calculate the magnification of the root tip.

3. Draw a diagram of the root tip, showing the separate layers of tissue. Labels are not necessary.

A scientist investigated the relationship between the distance of cells from the tip of a root, their mean volume, and the percentage of cells that were undergoing mitosis. The graph shows the results of the study.



Use the figure above to help you answer the following questions.

4. Describe how the percentage of cells undergoing mitosis varies as cells are sampled from increasing distances from the tip of the root.

5. With reference to both plotted lines, explain the results of the study.

Worksheet C: One moment in time

Figure 1. An afternoon at the fairground.

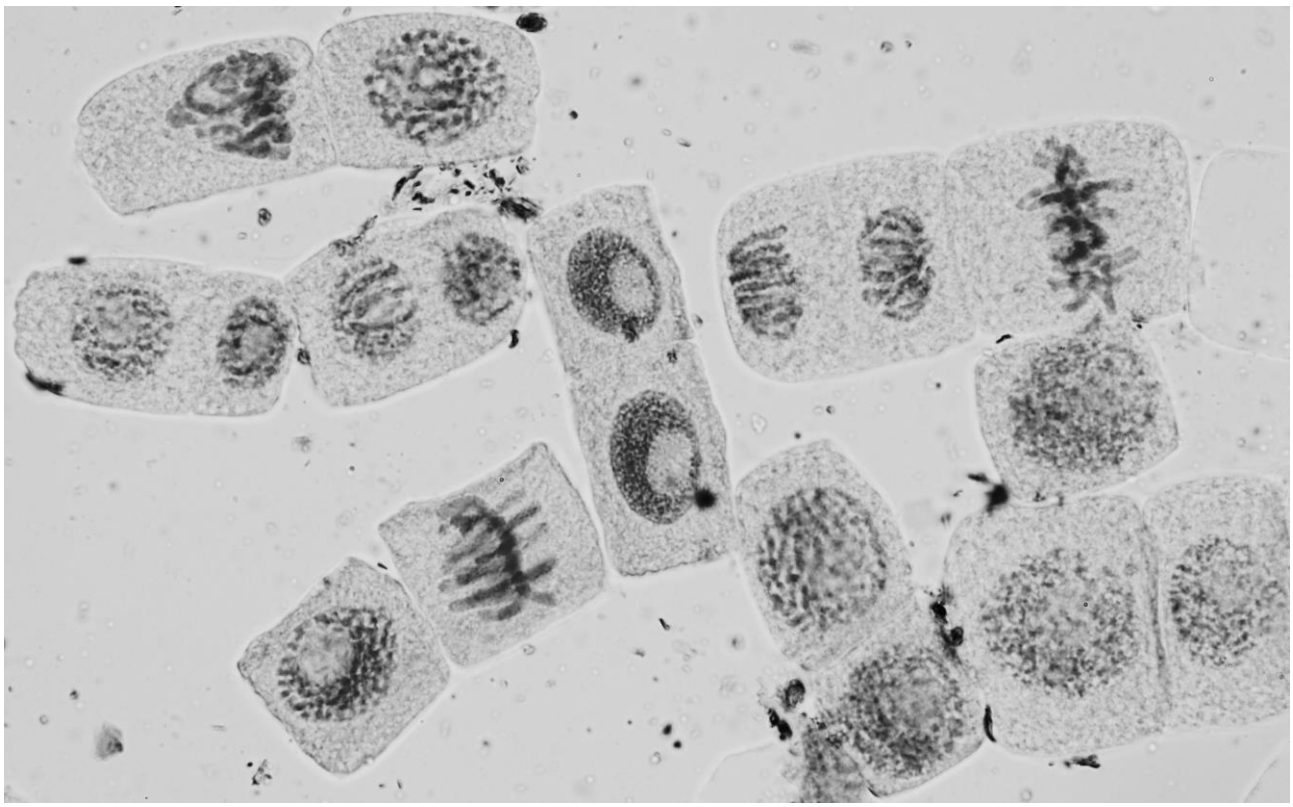


Figure 2. Image of cells taken from the root tip meristem tissue of a garlic clove. Magnification × 400.

Worksheet D: Planning a method

Problem	Suggested solution
Preparing plant cells for microscopy is a very unreliable process and single cells undergoing mitosis can be difficult to find.	
Cells undergoing mitosis are found in the meristem tissue in the tips of the roots.	
A polysaccharide called pectin binds together the cell walls of the root tip cells, which would prevent the tissue from breaking up into individual cells upon squashing.	
Untreated chromosomes are colourless and are invisible under a light microscope.	
Light cannot pass through thick tissue.	
Freshly prepared cells on a microscope slide can dehydrate and may not remain intact from one lesson to the next.	
Artefacts, such as fingerprints or air bubbles, can obstruct the view of cells down a light microscope.	
A magnification of at least × 400 must be used in order to view chromosomes with a light microscope.	

Worksheet E: The method

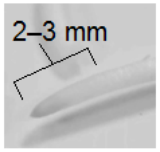
You will need to collect and set up the following apparatus and materials:

Apparatus or material	✓
• garlic clove with sprouting roots (on a toothpick)	
• 25 cm^3 of 1M hydrochloric acid	
• distilled water (in a wash bottle)	
• water bath set at 60°C	
• white tile	
• dissection scissors (very sharp)	
• scalpel (very sharp)	
• a pair of fine tweezers or a fine paintbrush	
• hot plate set at 40°C	
• watch glass	
• timer	
• 2 × 50 cm^3 beaker	
• 2 × dropping pipette	
• 1–2 cm^3 glycerol	
• 1% toluidine blue stain (in a dropper bottle)	
• microscope slide	
• 3 × coverslips	
• mounted needle	
• paper towels	
• light microscope	

Follow the instructions below very carefully.

1. Set your water bath to 60°C and the hot plate to 40°C (or check that they are at the correct temperature if they have already been set up).
2. Add 25 cm^3 of 1M hydrochloric acid to a 50 cm^3 beaker, and place this into a water bath set at 60°C for 10 minutes.
3. While the acid is warming, rinse the garlic roots with distilled water over a sink. Hold the garlic clove by the toothpick.
4. Once the acid is ready, suspend the garlic over the beaker of acid so that the roots are completely submerged in the acid.
5. Leave the garlic and beaker of acid in the 60°C water bath for 10 minutes.
6. After 10 minutes, remove the beaker from the water bath.

7. Over a sink, remove the garlic by holding the toothpick, then rinse off the acid using distilled water. Rinse for at least 5 seconds.
8. Hold the garlic clove over a white tile then use sharp scissors to cut off about 5 mm from three roots.
9. Use a scalpel to cut about 2–3 mm from the tip of each root.



10. Use a pair of fine tweezers (or a fine paintbrush) to gently transfer the small root tips to a watch glass.
11. Position all of the tips in the watch glass so that they occupy an area of about 1 cm^2. The tips should **not** be touching.
12. Add 1 drop of toluidine blue stain to cover all three root tips. Place the watch glass onto the hot plate set at 40°C. Leave for 10 minutes.

Do **not** let the stain dry out – remove the watch glass from the hot plate earlier if necessary, or add a small drop of distilled water.

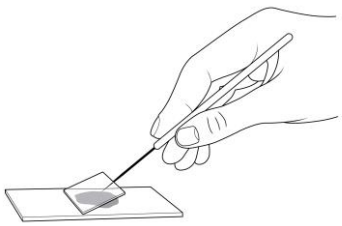
13. After 10 minutes, remove the watch glass from the hot plate.
14. Add 1 drop of toluidine blue stain over the thee root tips again. This time, **immediately** remove the stain with a dropping pipette and discard the removed stain into an empty 50 cm^3 beaker – this will act as your 'waste' beaker.
15. Add a few drops of distilled water to the root tips.
16. Use a clean pipette to remove the water from the root tips and discard it into your 'waste' beaker.

Make sure you do **not** contaminate the bottle of distilled water with stain. Do not use the wash bottle to remove stain from the sample and do not empty dirty water into the bottle.

17. Add a few drops of distilled water to the root tips and remove again using the pipette. If necessary, repeat until there is no more stain on the surface of the *watch glass* (the root tips however, should still be blue in colour).
18. Use the tweezers (or fine paintbrush) to gently transfer the root tips to a microscope slide.
19. Position each root tip along the length of the slide, about 2.5 cm apart.

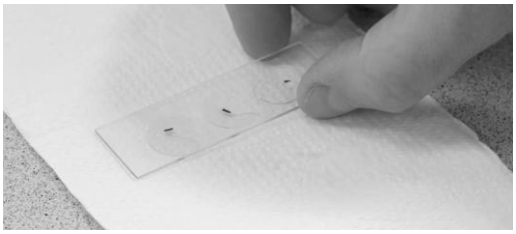
The distance between each tip does not have to be exact, you just need enough room so that you can fit a coverslip over each tip without the coverslips overlapping each other.

20. Add a small drop of glycerol onto each root tip.
21. Place a coverslip carefully onto each root tip. Place the coverslip at an angle over the sample and use a mounting needle to help you lower the coverslip down. This helps to avoid trapping too many air bubbles.



If you added too much glycerol in step 20, the root tissue will drift to the edge of the coverslip when you crush the sample later. Excess glycerol can be removed by dabbing with a paper towel at the edges of the coverslip.

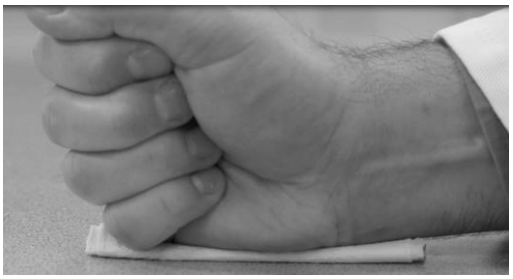
22. Fold a piece of paper towel in half lengthways.
23. Place the microscope slide onto the middle of the folder paper.



24. Carefully fold the paper towel over the slide, so that both sides of the slide are covered.

25. Carefully roll up the paper towel, making sure not to move the coverslips. Stop when the slide is completely wrapped in the paper towel.

26. Clench your fist into a ball. Place your fist on top of the folded paper towel. Push downwards to create a continuous downwards pressure. Do **not** twist or use sideways movements during this step. Make sure you do not twist or move your hand.



27. Carefully unwrap the slide from the paper towel and place it onto the stage of your light microscope.

If you need reminding of how to use a light microscope, see **Worksheet F**.

28. View one of the tips at a low magnification. You should be able to identify meristem cells: they are small, roughly circular or square-shaped cells with a large nucleus.

If there are too many air bubbles obscuring your view, you can add some water to the sample using a fine dropping pipette placed in one corner of the coverslip – this should push the air bubbles out.

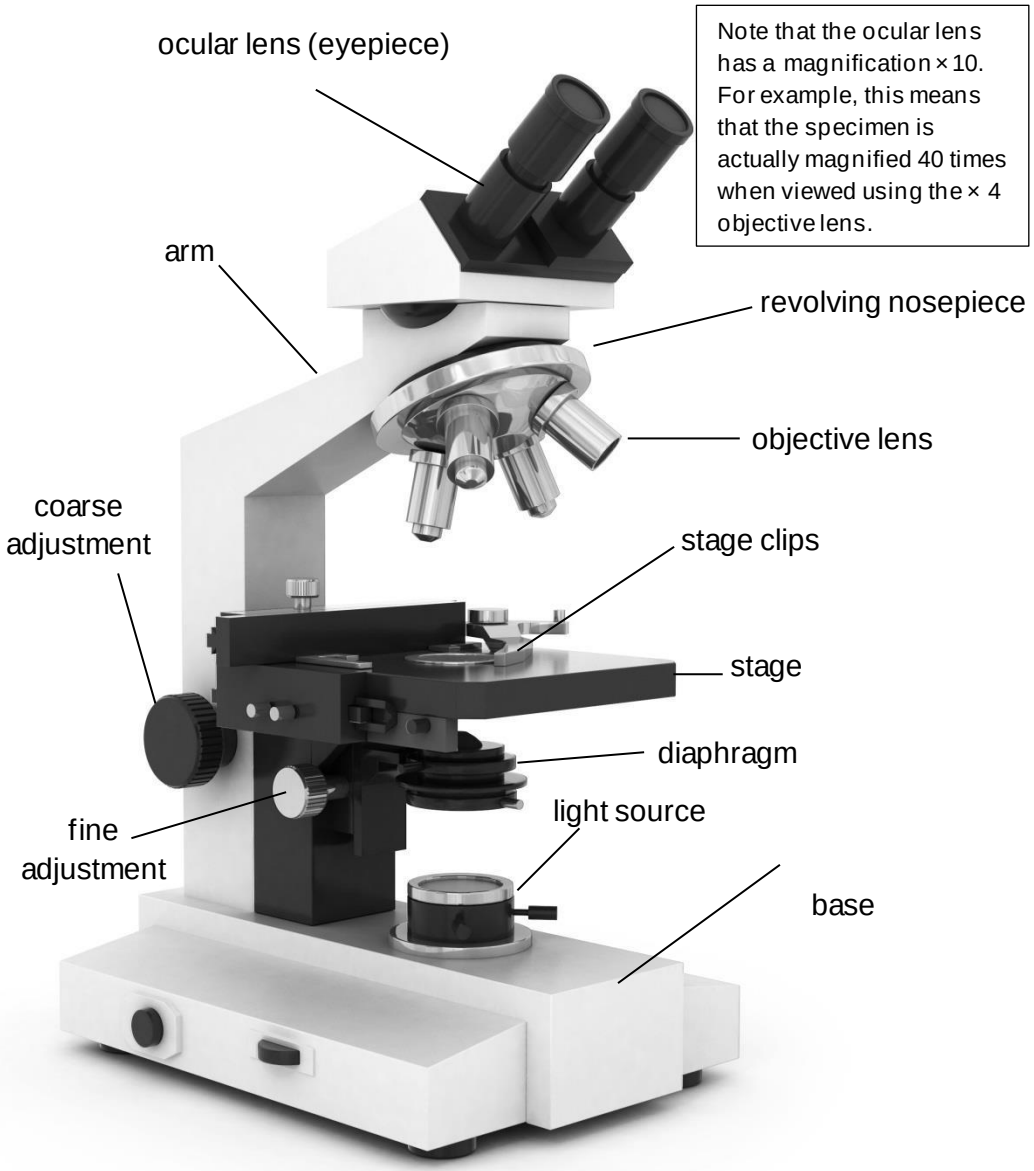
29. Once you have found some meristem cells in a layer of one only cell thick, view them under the highest magnification. You need a magnification of at least × 400 to see chromosomes and to distinguish between the phases of mitosis.

If one layer of cells is positioned on top of another, the view of the chromosomes will be obscured. So, if one root tip has not squashed well, look at your other tips. If all the samples have cells that are overlapping, wrap the slide and squash again.

30. Take your time to watch the specimen and identify which stages of mitosis you can see in the cells by observing the behaviour of the chromosomes. Record your findings on **Worksheet G**.

Worksheet F: How to use a 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; you can adjust the position of the diaphragm later to change the amount of light required.
4. Place the slide on the stage; secure it in place under the stage clips with the sample positioned over the central light hole.
5. Rotate the nosepiece so that the lowest power objective lens is in place. This is usually the × 4 magnification lens.
6. Raise the stage as far as possible using the coarse adjustment; be careful not to touch the slide with the objective lens. You should look at the microscope from the side when doing this.
7. Look through the eyepiece; move the slide if necessary to make sure the sample is in the field of view.
8. Bring the sample into focus using the fine adjustment, or moving the stage downwards by using the coarse adjustment.
9. Rotate the nosepiece so that a higher power objective lens is in place. It is not normally necessary to reposition the slide between lens changes.
10. Use the coarse and fine adjustments to adjust the focus until the cells are clearly visible.
11. When finished, lower the stage and rotate the nosepiece so that the lowest objective lens is in place. Remove the slide from the stage.



Note that the ocular lens has a magnification × 10. For example, this means that the specimen is actually magnified 40 times when viewed using the × 4 objective lens.

Worksheet G: Observing cells

Draw four cells in the spaces provided below. Include **labels** on your diagram and the **magnification** of your image. If possible, try to draw a cell for each of the following stages of mitosis: prophase, metaphase, anaphase and telophase. Describe the key features of the cell and the position of the chromosomes, and then decide which stage of mitosis they occupy.

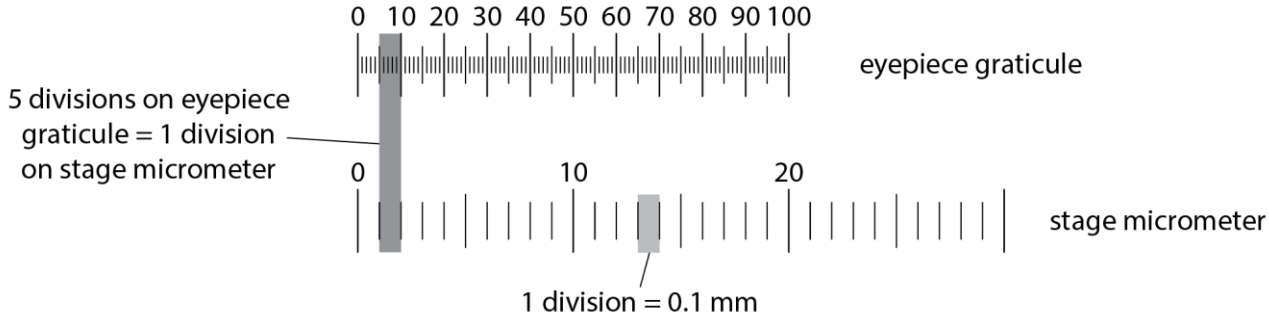
Stage of mitosis observed: I know it is this stage because:	Stage of mitosis observed: I know it is this stage because:
Stage of mitosis observed: I know it is this stage because:	Stage of mitosis observed: I know it is this stage because:

Worksheet H: Eyepiece graticules & stage micrometers

An eyepiece graticule is a transparent ruler with numbers, but no units. It is positioned inside the eyepiece. A stage micrometer is a microscope slide with a scale on its surface. It is required to calculate the length of the divisions on the eyepiece graticule at a particular magnification.

You can set up and use the eyepiece graticule and stage micrometer as follows:

1. Fit the eyepiece graticule to the eyepiece.
2. Place the stage micrometer onto the stage.
3. Line up the eyepiece graticule and the stage micrometer so that you can determine how many eyepiece divisions there are within 1 division on the stage micrometer, as shown below.



One division on the stage micrometer measures 5 divisions on the eyepiece graticule. Each division on the stage micrometer is 0.1 mm in length. Therefore, there are 5 divisions on the eyepiece graticule in 0.1 mm at **this** magnification.

4. To calculate the size of 1 division on the eyepiece graticule, the following calculation can be used:

$$1\text{ division on graticule} = \frac{\text{length of 1 division on stage micrometer}}{\text{number of graticule divisions equivalent to 1 division of stage micrometer}}$$

In the diagram above,

$$1\text{ division on graticule} = \frac{0.1}{5}$$
$$= 0.02\text{ mm } (20\ \mu\text{m})$$

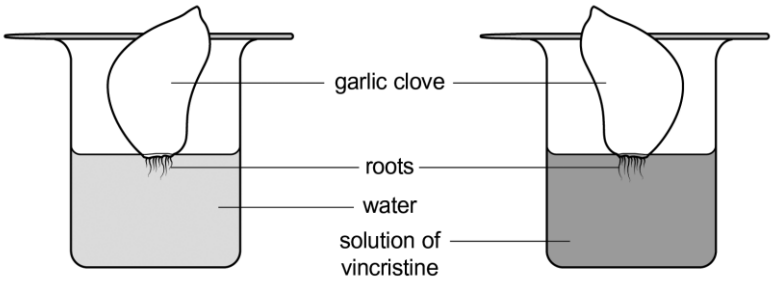
Therefore, when the stage micrometer is replaced with the slide containing the root tip squash, the size of the cells at this magnification can be calculated. For example, if a cell is 2.5 eyepiece divisions in length:

$$1\text{ eyepiece graticule division} = 20\ \mu\text{m}$$
$$\text{length of cell} = 2.5 \times 20\ \mu\text{m}$$
$$= 50\ \mu\text{m}$$

5. If you change magnification, you **must** recalibrate the eyepiece graticule by repeating step 3.

Worksheet I: An investigation involving mitosis

A scientist carried out an investigation into the effect of vincristine on the mitotic index of root tip meristem tissue. Vincristine is a chemical used to slow down the division of cancer cells. The scientist decided to incubate one clove of garlic in water and another clove of garlic in a solution of vincristine, as shown in **Figure 1**.



The scientist measured the effect of vincristine on the root tip tissue by calculating the mitotic index for both the untreated and treated garlic cloves. The mitotic index of a tissue sample is the proportion of cells that are undergoing mitosis at a given time, and it is expressed as a percentage. It can be calculated using the following formula:

$$\text{mitotic index} = \left(\frac{\text{number of cells undergoing mitosis}}{\text{total number of cells counted}} \right) \times 100$$

Figure 1 Experiment set-up.

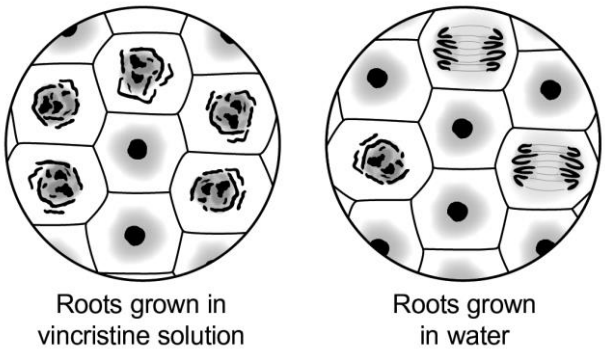


Figure 2 shows two fields of view of root tip meristem tissue from the garlic cloves.

1. Calculate the mitotic index of the root tissue grown in water to one decimal place. Only count cells in which you are able to **clearly** see the nucleus or chromosomes.

2. Calculate the mitotic index of the root tissue grown in vincristine solution to one decimal place. Only count cells in which you are able to clearly see the nucleus or chromosomes.

3. Explain how the scientist could improve the reliability of the mitotic index values obtained.

Vincristine stops cell division by affecting spindle fibres.

4. Describe the role of spindle fibres during mitosis.

5. The scientist concluded that vincristine prevents spindle fibre formation during mitosis. Explain how **Figure 2** provides evidence to support this conclusion.

Worksheet J: Student plan 1

The plan below was written by a student in response to the following question:

'How could the root tip squash method be used to investigate the concentration of vincristine that is most effective at inhibiting mitosis in root tip meristem tissue?'

The student has access to the same equipment and materials you used in your investigation but they were also given a 1.0 mg cm^{-3} solution of vincristine.

Firstly, two solutions of vincristine should be prepared, one of a low concentration and one of a high concentration. A control of tap water should also be used. The solution of low concentration can be prepared by mixing the original stock solution of vincristine with some tap water.

All three solutions are warmed to 30 °C and one clove of garlic is then placed into each of the solutions so that a section of the clove of the same length is touching the surface of each of the 3 solutions. These are left for 48 hours and all of the cloves are removed from the solutions at the same time for analysis.

The root tip squash method is then used to prepare a microscope slide for each of the garlic cloves and they are observed under a light microscope at × 400 magnification.

This is a low risk experiment, but care will be taken to handle sharp instruments carefully, and eye protection will be worn when handling the hot acid and the stain.

The number of cells undergoing mitosis in a field of view are counted for both slides and compared. These figures are plotted on a bar chart in order to compare the difference.

Worksheet J: Student plan 2

The plan below was written by a student in response to the following question:

'How could the root tip squash method be used to investigate the concentration of vincristine that is most effective at inhibiting mitosis in root tip meristem tissue?'

The student has access to the same equipment and materials you used in your investigation but they were also given a 1.0 mg cm^{-3} solution of vincristine.

Before the investigation, three further solutions of vincristine should be prepared by serial dilution, to give concentrations of $0.5 mg cm^{-3}$, $0.25 mg cm^{-3}$, and $0.125 mg cm^{-3}$. This can be done by mixing $5 cm^3$ of the stock solution with $5 cm^3$ of distilled water (to make the first dilution), mixing $2.5 cm^3$ of the stock solution with $7.5 cm^3$ of distilled water, to make the second, and $1.25 cm^3$ with $8.75 cm^3$ to make the third.

Next, eight garlic cloves are taken from four separate garlic bulbs (two from each bulb). Two garlic cloves are partly immersed in the original solution of $1.0 mg cm^{-3}$, and two garlic cloves from each of the other bulbs are immersed in the other three solutions. The garlic cloves are then left to take root for 48 hours.

Four slides are then prepared from the cloves in the four solutions by the root tip squash method. The number of cells undergoing mitosis are counted in three separate fields of view for each slide, and these numbers are then divided by the total number of cells in the field of view (and multiplied by 100) to give the mitotic index as a percentage. A mean is then calculated for each of the slides from the values, and this is plotted on a line graph against the concentration of vincristine.

Worksheet J: Student plan 3

The plan below was written by a student in response to the question:

'How could the root tip squash method be used to investigate the concentration of vincristine that is most effective at inhibiting mitosis in root tip meristem tissue?'

The student has access to the same equipment and materials you used in your investigation but they were also given a 1.0 mg cm^{-3} solution of vincristine.

From the original solution, four further solutions of vincristine should be prepared, of concentrations 0.09, 0.08, 0.07 and 0.06. A control should also be used, which is distilled water.

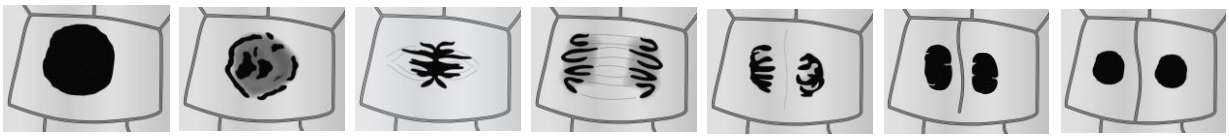
Into each of these six solutions, three garlic cloves taken from the same large bulb are placed (total: 18 cloves). During the period of incubation, the temperature of the solutions containing the cloves is kept constant at 25°C.

The root tip squash method is then used to prepare six slides on which there are three samples of roots each. Three fields of view are selected at random for each of the root samples, and the number of cells undergoing mitosis in each of these is then divided by the total number of cells visible in the field of view. A mean and standard error is then calculated for the data obtained for each dilution, and 95% confidence interval error bars are drawn on a plotted graph to provide a measure of the significance of the differences between the mean values for each solution. If the error bars between any two points overlap, then there is unlikely to be a significant difference between the sample means. However, a chi-squared test would be needed to investigate the actual significance of any differences between the mean values.

Teacher Instructions 1: The order of stages in mitosis

On each of the following pages there is an image of a cell undergoing mitosis. Print out one copy of each image, preferably onto A3 paper, and laminate. There is a space provided on the sheets to add the name of the stage of mitosis at the end of the activity. These will be used in the start activity in the *Briefing lesson.*

The correct order of images to show the order of mitosis is shown below.



interphase / early prophase | prophase | metaphase | anaphase | early telophase | late telophase | interphase / early prophase

Learners should be challenged to describe the key events that are characteristic of each stage of mitosis, e.g.:

1. **In interphase/early prophase**, the nucleus appears homogeneous in colour and texture and is darkly stained.

2. **In prophase**, the chromosomes begin to condense and start to become visible in the cell. The nuclear membrane begins to disintegrate.

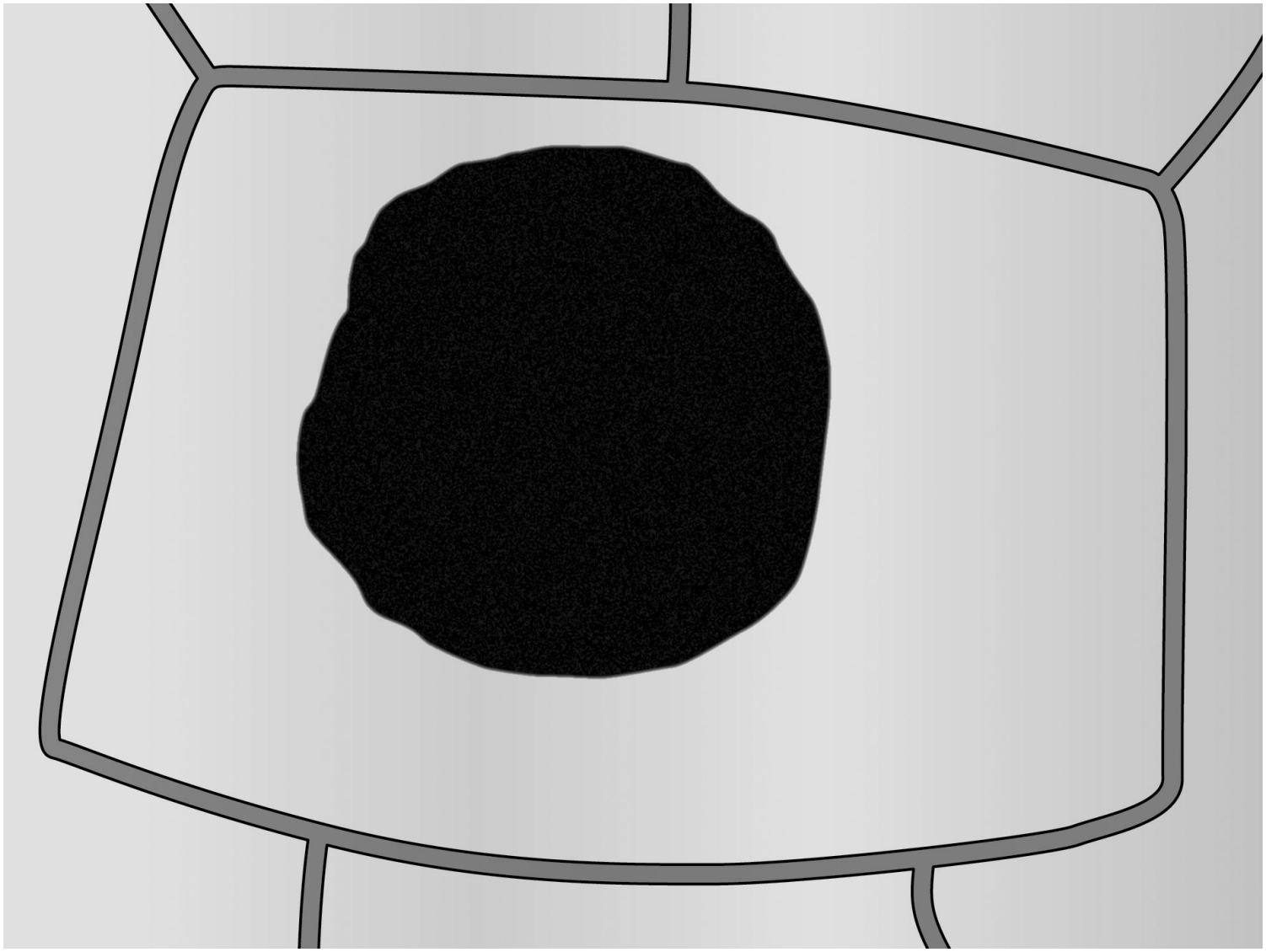
3. **In metaphase**, the chromosomes line up along the equator of the cell. Spindle fibres radiate from the poles of the cell and attach to the centromeres, the structures that hold together the two sister chromatids.

4. **In anaphase**, the spindle fibres contract and pull the sister chromatids apart and to opposite poles of the cell.

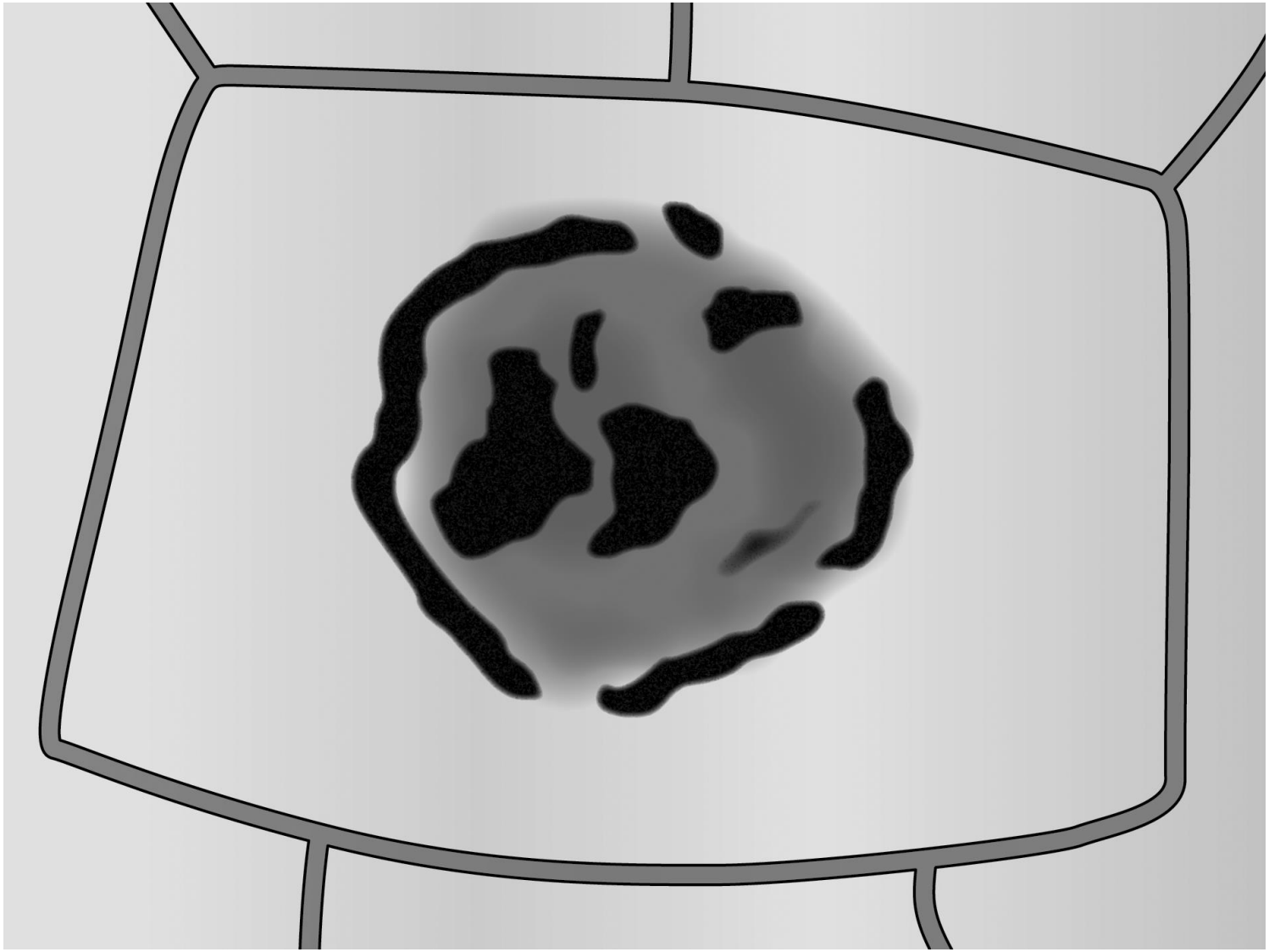
5. **In early telophase**, a nuclear membrane begins to form around each set of separated sister chromatids, now called chromosomes.

6. **In late telophase**, the cytoplasm begins to divide between each set of separated sister chromatids in the process of cytokinesis. A cell plate also forms in order to lay down a new cell wall.

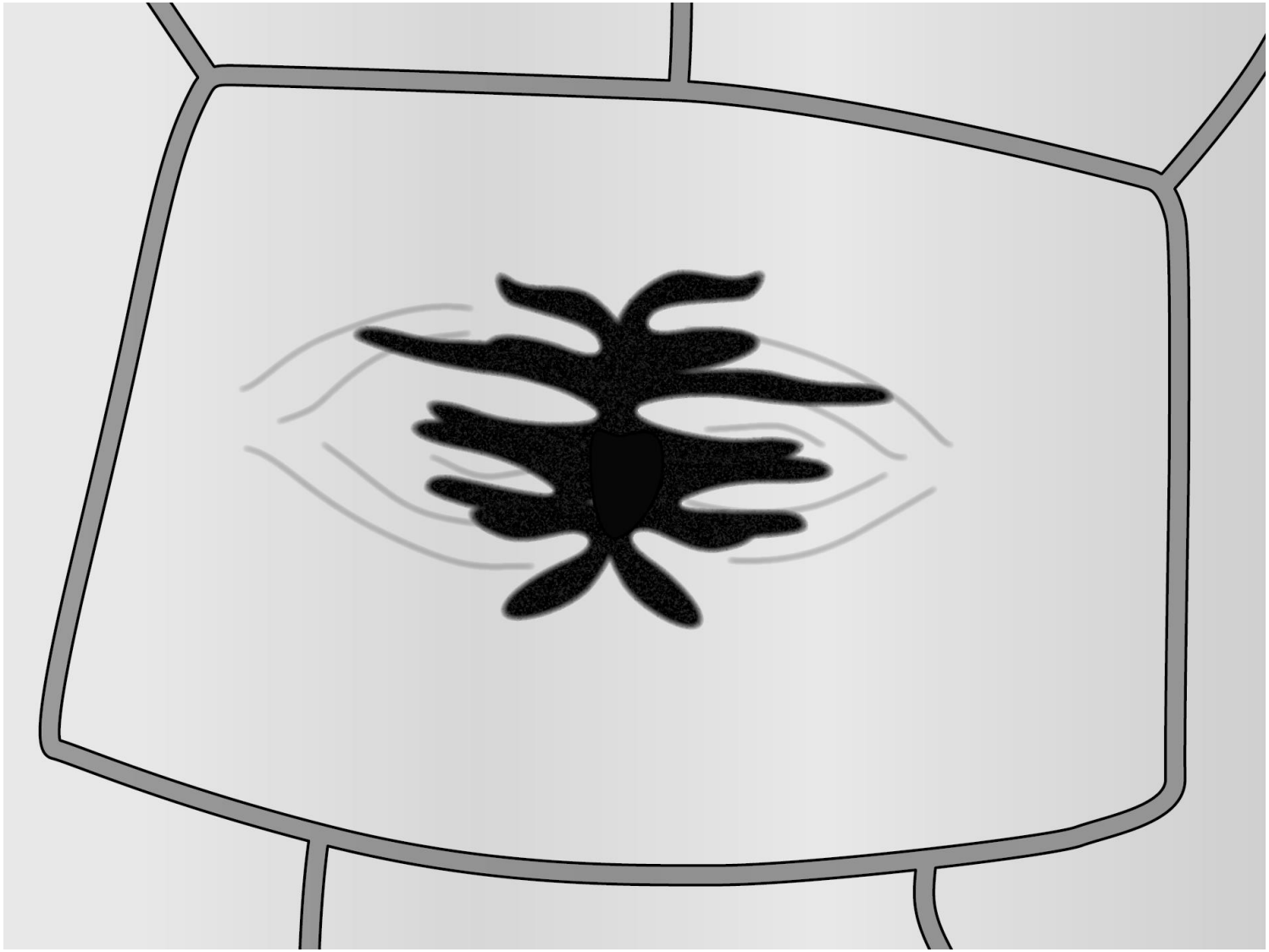
7. The cycle begins again as the cells enter **interphase/early prophase**.



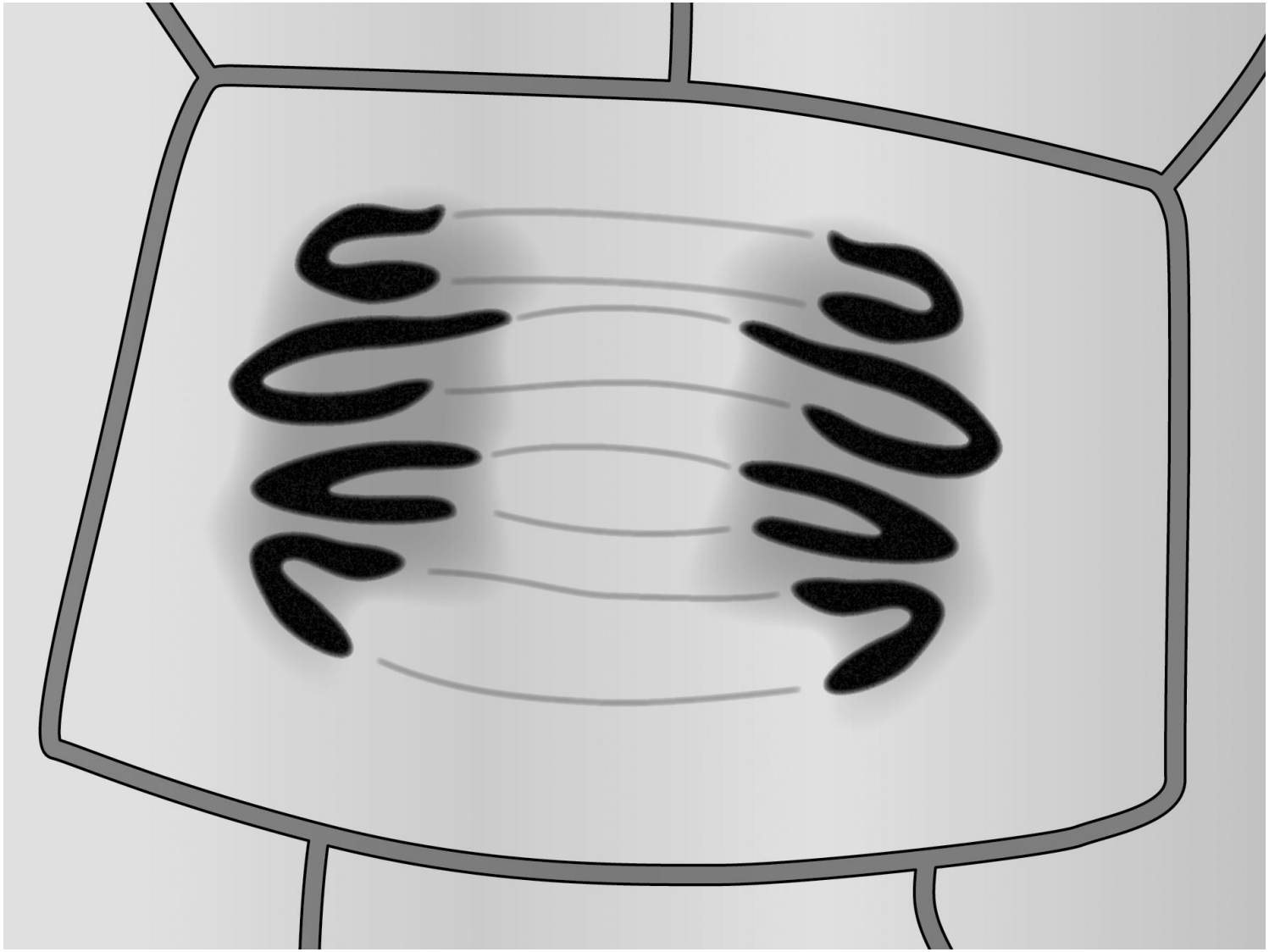
Stage of mitosis:



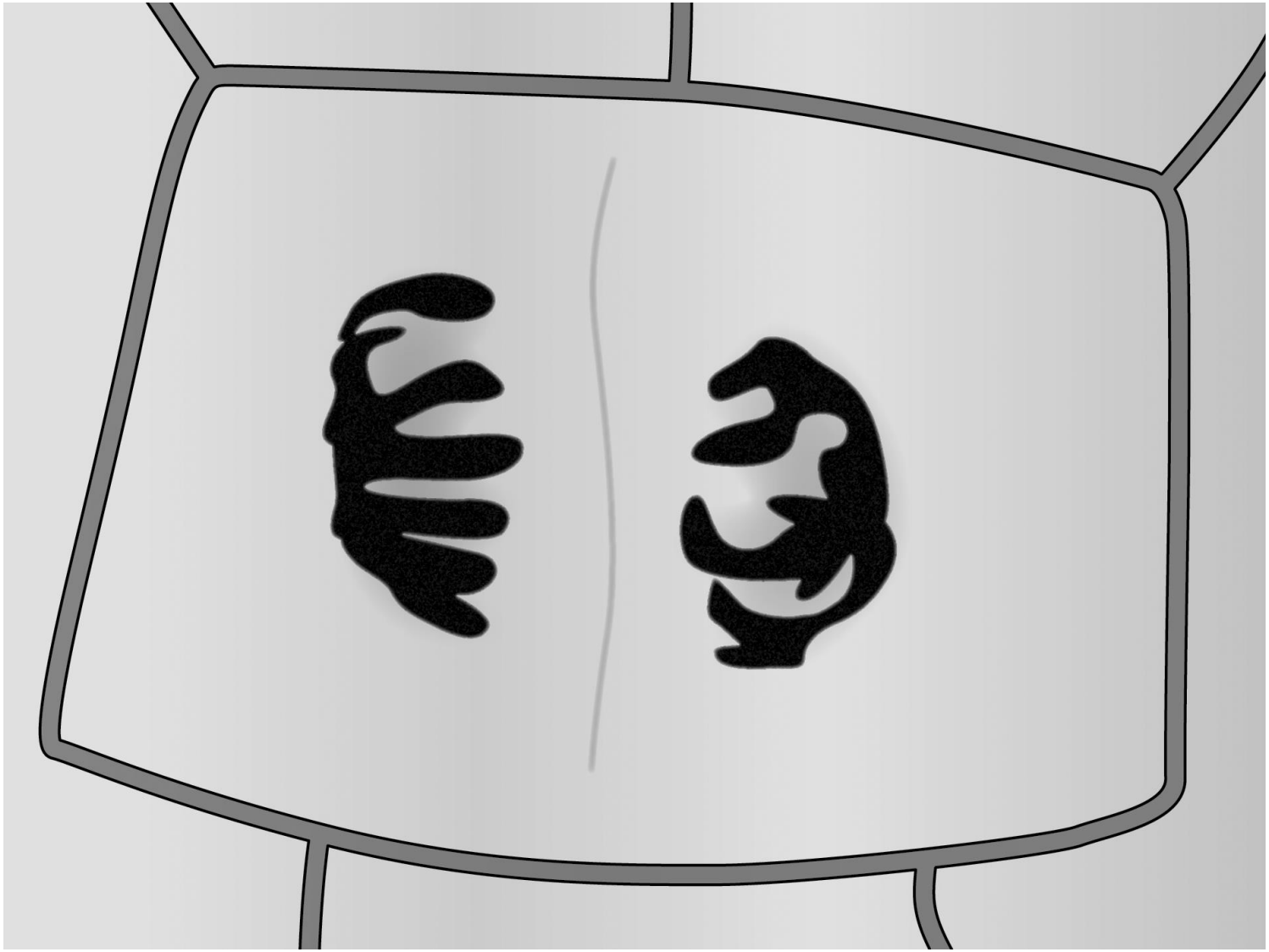
Stage of mitosis:



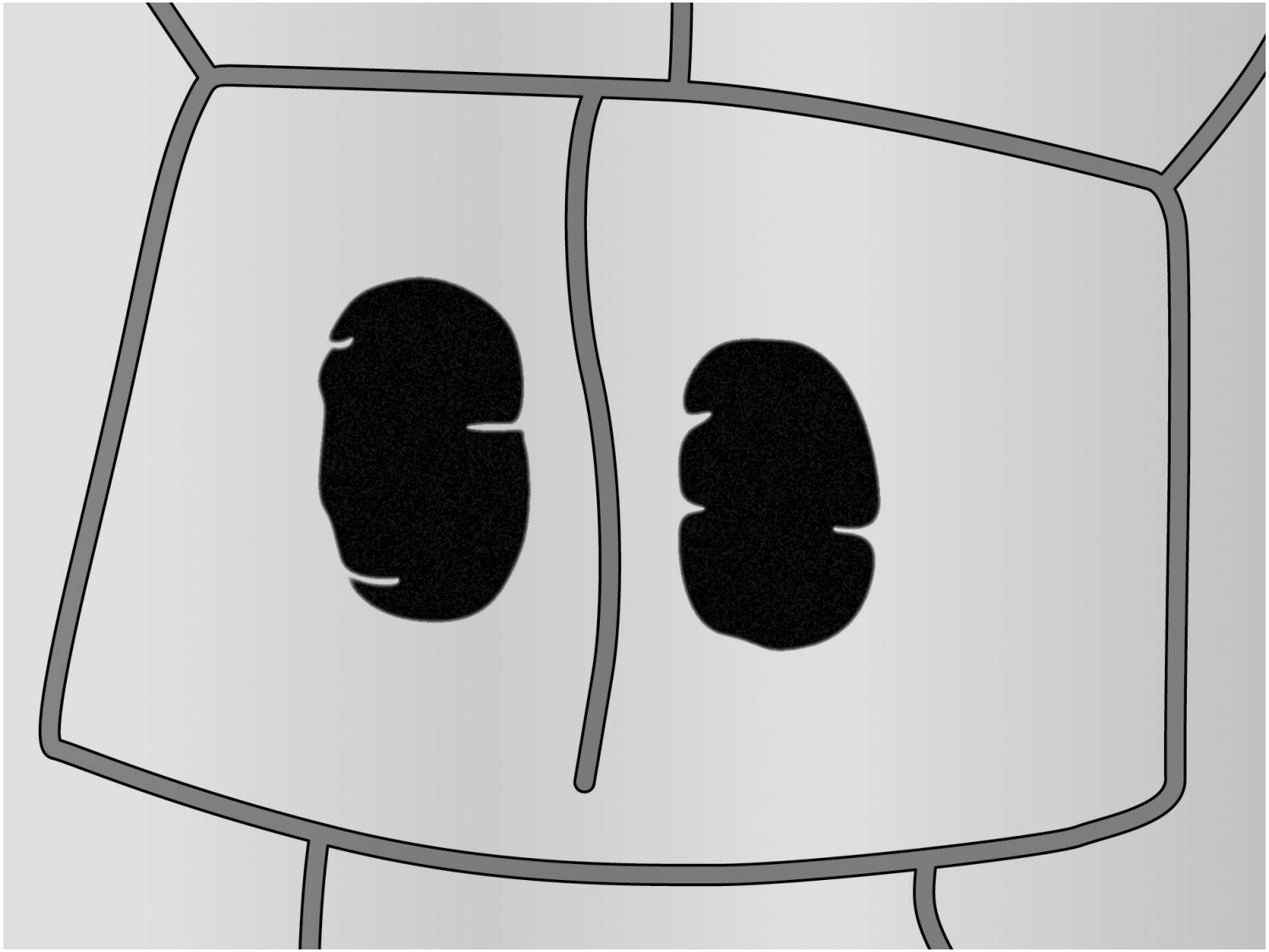
Stage of mitosis:



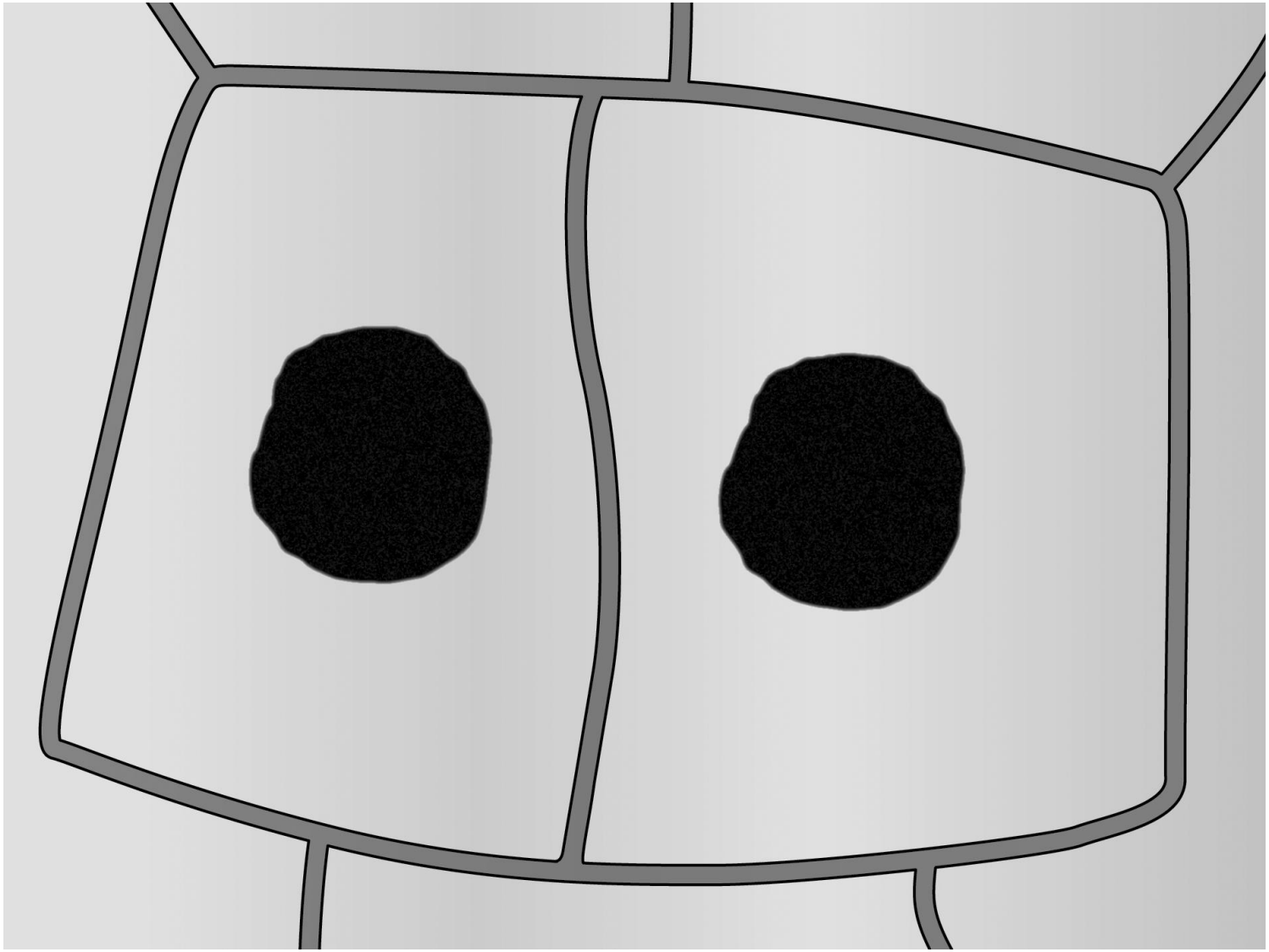
Stage of mitosis:



Stage of mitosis:



Stage of mitosis:



Stage of mitosis:

Teacher Instructions 2: Circus support

Notes for Task 1
Photocopy and cut out **Figure 1** so that there is one per group; then put the copies into an envelope and place it on the appropriate station.

Notes for Task 2
Photocopy and cut out **Figure 2** so that there is one per group; then put the copies into an envelope and place it on the appropriate station.

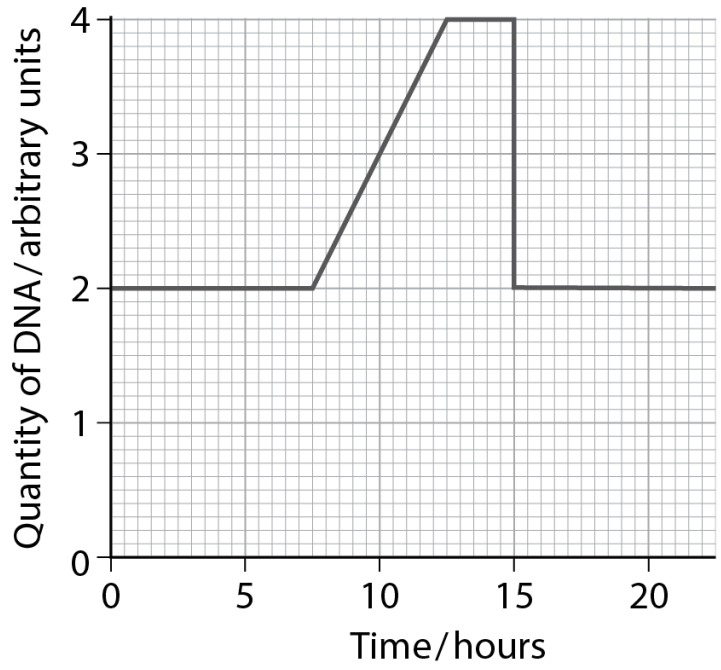


Figure 1

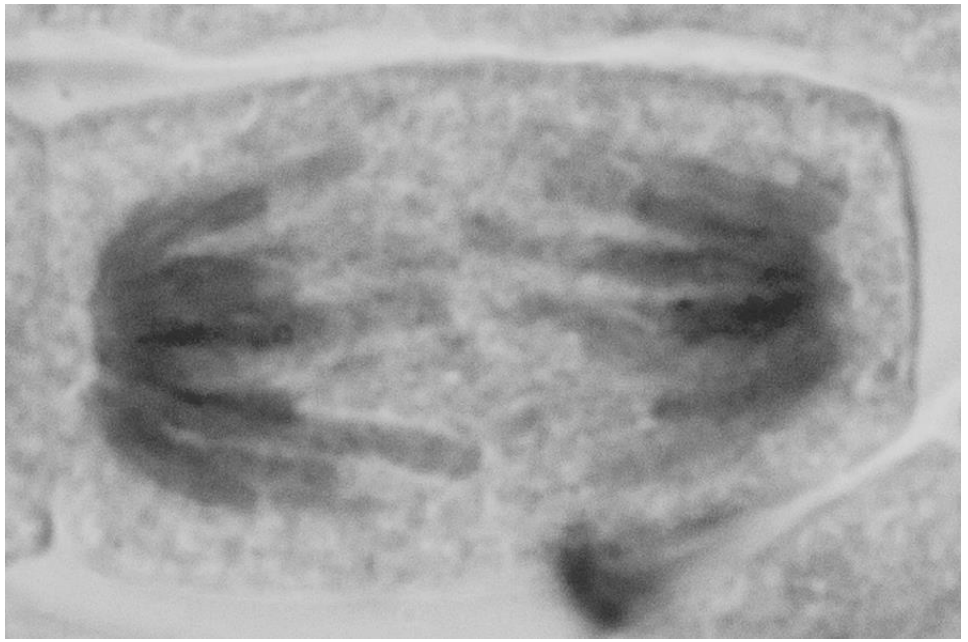


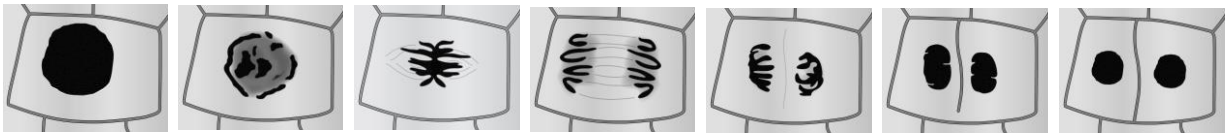
Figure 2

Notes for Task 3

In this task, learners will need to use the following formula:

$$\text{magnification} = \frac{\text{measured length}}{\text{actual length}}$$

Print the seven images from Teacher instructions 1 (thumbnails shown below) onto A4 paper.



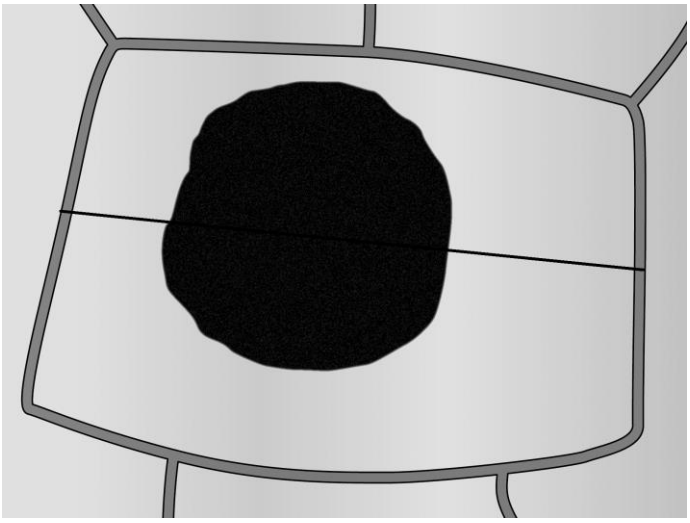
interphase / early prophase | prophase | metaphase | anaphase | early telophase | late telophase | interphase / early prophase

For 3–4 images, write 'magnification 3500 ×' onto the white space around the image. Learners should calculate the actual size of the cell by measuring the length of the cell in the image in millimetres and dividing this by the magnification provided. Their answer should be converted into micrometres by multiplying their answer by 1000.

For the remaining 3–4 images, add a scale bar measuring 35 mm to the white space around the image. Label the scale bar with '10 μm'. Learners should calculate the magnification of the image by dividing the length of the scale bar (35 mm) by the measurement label (10 μm, converted to 0.01 mm), giving a value of 3500 ×. Alternatively, you can give the actual length of the cell (~48 μm) and ask them to use the above formula to calculate the magnification.

Alternatively, you can add the relevant information onto the versions of the images stuck on the wall using sticky labels.

Note: the above values are based on the linear length of the cells being about 17 cm across the middle (see below) when printed on A4 paper; you might need to adjust the values depending on the size of the images once they are printed.



Worksheet A: Answers

Task 1:

Example description
The quantity of DNA remains at 2 units from 0 to 7.5 hours, but then rises to reach 4 units at 12.5 hours. It then remains at 4 units for another 2.5 hours until 15 hours, until it falls immediately to 2 units and remains at this level (the next cycle begins).

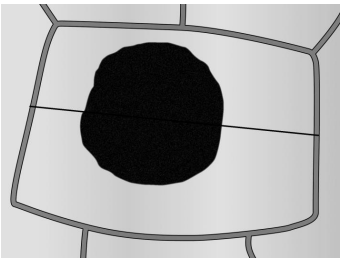
Example explanation (extension activity)
The replication of DNA (in interphase) takes 5 hours, during which the quantity of DNA in the cell doubles as chromosomes become sister chromatids. Between S phase and mitosis, the DNA content of the cell is twice that of a normal diploid cell. The division of this DNA between two daughter cells, at time = 15 hours, is almost immediate as telophase occurs very quickly.

Task 2:

Example description
The cell does not have a nucleus and is rectangular in shape. There are around 4 sister chromatids visible, which appear as strands that are being pulled to the opposite poles of the cell (towards the shorter sides of the rectangle). The sister chromatids have completely separated from one another and there is a clear space between them.

Example explanation (extension activity)
Spindle fibres, which attach to the centromeres of chromosomes at metaphase, begin to contract at anaphase and pull the sister chromatids apart to opposite poles. This allows for the DNA in the parent cell to be equally shared between the daughter cells.

Task 3:
Note: the below values are based on the linear length of the cells being about 17 cm across the middle when printed on A4 paper; you might need to adjust the values depending on the size of the images once they are printed.



1. Learners should calculate the actual size of two cells on which the magnification (3500 ×) has been written. To do this, they should:

- measure the length of the cell on the print out using a 30 cm ruler in millimetres
- divide the measurement of the cell in mm by the magnification (3500); this will give a value in millimetres
- convert the value for the actual size into μm by multiplying by 1000; the exact value that learners will obtain will vary, but should be around 48 μm.

2. Learners should calculate the magnification of the images of two cells on which you have drawn a scale bar of length 35 mm and labelled '10 μm'. To do this, they should:

- measure the length of the scale bar with a 30 cm ruler in millimetres
- convert the stated scale bar measurement (10 μm) into millimetres by dividing by 1000
- divide the actual length of the scale bar in mm by the stated measurement in mm to give a value for the magnification of 3500 ×.

Task 4:

1. Learners should identify the following mistakes made by the three students in their diagrams:

STUDENT 1	STUDENT 2	STUDENT 3
Lines are broken rather than continuous	Shading has been used	More than one cell is drawn
Sister chromatids are not in proportion to the cell – they are too small (inaccurate representation)	Not all sister chromatids are drawn (one is missing from the right-hand side)	Sister chromatids are not in the correct position relative to each other and the equator of the cell (inaccurate representation)
Key labels are missing (e.g. cell wall, sister chromatids)	Label lines have not been drawn using a ruler	Some labels are incorrect (e.g. cell wall label points to the cell membrane; chromosomes should be sister chromatids) and others are missing, e.g. cell wall

2 Learners' diagrams should not make any of the mistakes listed in the table above.

When drawing scientific diagrams through a microscope, learners should:

- use continuous lines
- not include shading
- only draw the focus cell (one cell)
- all contents of the cell should be in proportion to the cell
- structures should be labeled
- label lines should be drawn using a ruler
- all components should be drawn (in this case, that refers to four sister chromatids on each side of the cell)
- contents of cell should be in correct position relative to each other
- use as much of the available space as possible.

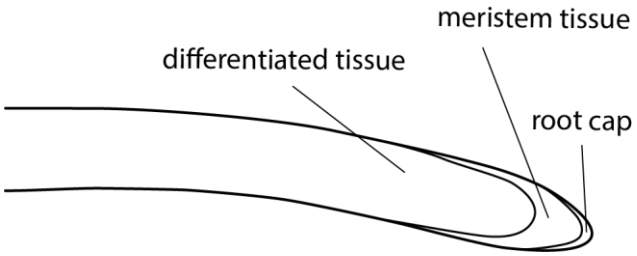
Worksheet B: Answers

1. B. This region contains meristem tissue that contains highly mitotic stem cells with a short period of interphase.

2. $$\text{magnification} = \frac{\text{measured length of scale bar}}{\text{stated length of scale bar}}$$

   $$\text{magnification} = \frac{10\ (\text{mm})}{0.1\ (\text{mm})}$$

   $$= 100\times$$

3. 

4. At the very tip of the root, in the root cap, very few cells are undergoing mitosis. However, just behind the cap, the majority of cells are dividing in the meristem tissue. As distance from the tip increases further, the number of cells undergoing mitosis falls gradually, and then more suddenly, until the value reaches zero. Beyond this point, all cells have differentiated and do not undergo mitosis.

5. At the root tip behind the cap, meristem tissue exists which is the site of cells undergoing rapid mitosis. This produces daughter cells that move backwards, further away from the root tip as the root grows. As these daughter cells move further from the tip, they stop dividing, and begin to elongate, as their vacuoles gain water by osmosis. This results in their volume increasing.

Worksheet D: Answers

Problem	Expected solution
Preparing plant cells for microscopy is a very unreliable process and single cells undergoing mitosis can be difficult to find.	*At least three root tips should be prepared on the slide to increase the likelihood that some cells are successfully prepared and have visible chromosomes. It effectively provides an increased area to search for cells undergoing mitosis.*
Cells undergoing mitosis are found in the meristem tissue in the tips of the roots.	*The very end 2–3 mm of the root tips should be removed and used in this procedure because cells further towards the clove will not be undergoing mitosis. Once the slides have been prepared, a low power magnification should be employed to identify small, roughly circular or square-shaped cells with a large nucleus, before increasing the magnification to identify cells undergoing mitosis and to view the behaviour of chromosomes.*
A polysaccharide called pectin binds together the cell walls of the root tip cells, which would prevent the tissue from breaking up into individual cells upon squashing.	*The root tips should be treated with hot, 1M hydrochloric acid to hydrolyse the pectin between the cell walls. This would allow the cells to be separated for the purpose of squashing the tissue and viewing them.*
Untreated chromosomes are colourless and are invisible under a light microscope.	*A stain should be applied to the cells such as toluidine blue to make the chromosomes visible. The stain should be washed away from the surroundings of the tissue, to ensure a clear distinction between the genetic material and the rest of the cell on the slide. During the process of staining, heating the tissue to 40 °C intensifies the stain and allows it to better penetrate the cells.*
Light cannot pass through thick tissue.	*The slide, wrapped in a number of paper towels, should be squashed under a coverslip to obtain a single layer of cells. Continuous downward pressure should be applied to the slide using a clenched fist. There should be no twisting or sideways movement as this could damage the glass or the coverslip.*
Freshly prepared cells on a microscope slide can dehydrate and may not remain intact from one lesson to the next.	*A drop of glycerol should be added to the tissue before the coverslip is applied. This minimises the risk of the tissue becoming dehydrated.*
Artefacts, such as fingerprints or air bubbles, can obstruct the view of cells down a light microscope.	*Microscope slides and coverslips should be held carefully by the edges to prevent getting fingerprints on them. The coverslip should be placed down slowly on the microscope slide using a mounted needle, in order to support it as it is lowered at an angle over the tissue. Trapped air bubbles can be removed by flushing water under the coverslip after squashing; a fine pipette is placed next to one corner and water is pushed out.*
A magnification of at least × 400 must be used in order to view chromosomes with a light microscope.	*An objective lens of × 40 should be used. This is magnified × 10 by the eyepiece lens to give a total magnification of × 400.*

Worksheet G: Answers

Learners' own drawings.

For each drawing, check that:

- the correct stage of mitosis has been identified
- labels and magnifications are correct
- a sharp pencil has been used to give finely drawn lines
- lines are clear, sharp and unbroken
- shading has **not** been used
- the learner has made the most of the available space to show all the features observed in the specimen.

Worksheet I: Answers

1. Mitotic index = (5 ÷ 9) × 100 = **55.6%** (to 1 d.p.)

2. Mitotic index = (3 ÷ 9) × 100 = **33.3%** (to 1 d.p.)

3. Only 9 cells were counted from each garlic clove. To improve the reliability, the scientist should select, at random, a number of fields of view (minimum 3 for each treatment) in which to identify cells and calculate their mitotic index. By calculating the mean of these values, the effect of anomalous results will be minimised and the reliability of the data improved.

4. Spindle fibres attach to the centromeres of chromosomes during metaphase and contract to pull the sister chromatids apart to opposite poles of the cell during anaphase.

5. There are many cells in the tissue treated with vincristine that have been arrested in prophase. This is in keeping with the absence of spindle fibre formation, as the chromosomes cannot align on the equator and cannot subsequently be pulled apart into separate sister chromatids in anaphase.

Worksheet J: Example responses

Listed below are some of the criticisms of the three students' work that learners should mention. This can also be used to prompt further discussion.

Plan 1:

- An insufficient number of concentrations of vincristine are investigated. This would not provide enough information to plot an accurate graph to study a relationship between the independent and dependent variables.
- The actual concentrations of the vincristine solutions used are not stated. The person following would not know what is 'high' and 'low' and how to prepare these.
- Tap water, rather than distilled water, is used to prepare the vincristine dilutions. There may be substances in tap water, not present in the vincristine solutions, which may have an effect on mitotic index.
- Only one clove of garlic is used for each solution and (we assume) only one count of cells is performed for each microscope slide. This means that any anomalous data would not be identifiable, and a mean value could not be calculated. The reliability of the data yielded from this study would be very poor.
- Although the solutions are all initially warmed to the same temperature, they were not incubated at this temperature for the duration of the incubation period. There is no way of knowing that the different solutions will have remained at the same temperature for the duration of the study.
- The mitotic index is not calculated; instead, the number of cells undergoing mitosis is recorded, which cannot be legitimately compared between slides. This is because the number of cells present in separate fields of view may differ.

Plan 2:

- The method indicates that a serial dilution method will be used to prepare the different concentrations of vincristine, but the plan describes a proportional dilution method.
- A control (garlic clove placed into distilled water) is not used. This is needed in order to remove the effect of the independent variable, vincristine concentration.
- The two garlic cloves that are placed into each solution are from different bulbs; it would make the investigation more valid if all cloves used were from the same bulb to minimise any genetic variation that could influence mitotic index between different garlic plants.
- Some key variables have not been controlled to make the investigation valid, e.g. temperature, the length of the basal plate of the clove that is immersed, and so on. This means that we cannot be sure if any observed effect is due to the independent variable.
- There is no mention of using standard deviation, standard error, or plotting error bars. This means that there is no way to ascertain the reliability of the mean values of the mitotic index for each solution.

Plan 3:

- Although four further solutions are prepared, the range of their dilutions is too narrow to provide enough information to plot an accurate graph to study a relationship between the independent and dependent variables.
- It is not clear how the suggested concentrations of vincristine would be prepared; another individual could not follow the method accurately.
- Units are not provided for the concentrations of the five solutions, which means that another individual following the method would not know what concentrations to use.

- The temperature is kept constant for the duration of the investigation, but the time for incubation and how temperature will be maintained (e.g. by using a thermostatically controlled water bath) is not described.
- How the fields of view are selected for counting at random is not described.
- A student's *t*-test, rather than a chi-squared test, would be a more appropriate statistical test to apply to this data as mean values are being compared.

Cambridge Assessment International Education
The Triangle Building, Shaftesbury Road, Cambridge, CB2 8EA, United Kingdom
t: +44 1223 553554 f: +44 1223 553558
e: info@cambridgeinternational.org www.cambridgeinternational.org