OBSERVING MITOSIS

Purpose

- To prepare some slides of actively dividing plant tissue.
- To observe the stages of the cell cycle in living tissue.
- To determine the duration of the stages of mitosis in relation to the whole cell cycle.
- To develop practical skills.

Preparing the cells

To see mitosis in action you need to look at living cells. Garlic bulbs grow roots that have actively dividing cells in their tips. Each cell has only eight chromosomes so it is relatively easy to see the chromosomes once they have condensed.

In order to see the chromosomes inside the cells, the cells must be separated and spread out into a layer that is ideally just one cell thick. Plant cells are glued together by a middle lamella of pectins. Hydrochloric acid will break down these pectins allowing the cells to be separated. Follow the procedure in Methods 1 or 2 to stain chromosomes. Your teacher will guide you on which method to use. Before you start, read the method carefully and consider any safety issues and how you will minimise any risks.

Interpreting what you see on your cell preparation

Examine your preparation carefully for cells undergoing different stages of mitosis. Identify the different stages by comparison with labelled pictures or photographs of cells during mitosis. Bear in mind that mitosis is a dynamic process so cells may have been fixed in transition from one stage to the next – you will have to interpret what you see. Follow the steps below to help you record and interpret your results.

1 Identify cells in the following stages of mitosis: interphase, prophase, metaphase, anaphase and telophase. Draw one cell to illustrate each stage. Your drawings will be simple outlines of the cells and the groups of chromosomes in them as few other structures will be visible. Aim to show the relative sizes and positions of the chromosomes in the cell accurately. Annotate to describe what is happening. See Practical Skills Support Sheet 8 – using a microscope – for guidance on biological drawing.

2 Count the number of cells in the area visible under the microscope when viewed at ×400 (the field of view). Count the number of cells in each stage of mitosis. Record your results in an appropriate table.

3 Calculate the percentage of the cells in each stage of mitosis. Rank these values from highest to lowest. Given that your preparation freezes the process of mitosis at one point of time, what do these values suggest to you about the length of time a cell spends in each stage of mitosis? Explain how you arrive at your conclusion.

4 If a group of cells is dividing rapidly, a high proportion of the cells will be undergoing mitosis. A group of cells that is not dividing will have all cells in interphase of the cell cycle. The amount of cell division occurring in a tissue can be quantified using the mitotic index. Using the formula below, calculate the mitotic index for your root tip. If you have time, compare this value with the mitotic index of an area of cells away from the tip and comment on your findings.

$$\text{Mitotic index} = \frac{\text{number of cells containing visible chromosomes}}{\text{total number of cells in the field of view}}$$

The mitotic index is useful in studies of cell division in many different types of tissues, for example in examining tumour growth in cancer patients.

5 Using a stage micrometer and eyepiece graticule, make appropriate measurements to allow you to compare the size of interphase cells with those that are undergoing cytoplasmic division. See Practical Skills Support Sheet 9 – size and scale – for information on the use of a stage micrometer and eyepiece graticule. Comment on your findings.

Evaluation

After completing the practical work, evaluate the method used and results obtained. In this evaluation you should:

- comment on the suitability of the procedure
- describe and explain any changes you made to the method provided
- explain the safety precautions taken during this practical
- discuss the quality of your results, including comments on the validity of the results, and the repeatability, accuracy and precision of any measurements made
- discuss any limitation of the method and apparatus, and suggest what modifications could reasonably be made to the procedure or apparatus to improve your findings.

Method 1 Using toluidine blue stain

SAFETY

1 M hydrochloric acid is an irritant. Wear eye protection, lab coats and disposable gloves.

Toluidine blue is harmful if ingested and will also stain skin and clothes.

Wear lab coats and disposable gloves.

Always use the fine forceps to move the root tip sample to/from solutions.

Be aware of the risk of using microscopes where direct sunlight may strike the mirror.

YOU NEED

- Garlic roots
- 1 M hydrochloric acid
- Toluidine blue stain
- Cold distilled water
- 2 watch glasses or small sample tubes
- Hollow glass block or small sample tube
- Pipettes (and pipette fillers) or small measuring cylinders
- Microscope slides and coverslips
- Pair of fine forceps
- Filter paper or soft tissue paper
- Microscope with magnifications of ×100 and ×400
- Fine scissors

Procedure

1 Cut off about 5 mm from several root tips of some growing garlic roots using fine scissors. Choose root tips that are white and have a firm, rounded end; tips that are turning brown will give poor results.

2 Put the root tips into a hollow glass block or small sample tube containing 2 cm^3 1 M hydrochloric acid for exactly 5 minutes.

3 Put the root tips in a watch glass containing approximately 5 cm^3 cold water. Leave the root tips for 4–5 minutes, then dry them on filter paper. Take care – the root tips will be very fragile.

4 Transfer one of the root tips to a clean microscope slide.

5 Gently break up the root tip with a mounted needle (this is called maceration). Add one small drop of toluidine blue and leave to stain for 2 minutes.

6 Cover with a coverslip and blot firmly with several layers of tissue or filter paper. Press gently to spread the root tip, or tap gently on the coverslip with the end of a pencil.

7 View under the microscope (×400 magnification) and look for cells with visible chromosomes. If cells are overlapping, squash the slide again between two wads of filter paper. Avoid lateral movement of the coverslip.

8 Look for regularly shaped, actively dividing cells. DNA stains dark blue with toluidine blue stain so you should be able to see blue groups of chromosomes against a paler background.

9 If your preparation is not very successful, repeat with some of the other root tips from step 3. Try to adjust your procedure to remedy the problem; for example, if your cells are over- or under-stained, adjust the time they are left in the stain.

Method 2 Using orcein ethanoic stain

SAFETY

Wear eye protection, lab coats and disposable gloves throughout.

1 M hydrochloric acid is an irritant.

Orcein ethanoic stain is corrosive, irritant, causes burns, has an irritating vapour and stains. Avoid contact with skin. If contact does occur, wash the area thoroughly with water for 10 minutes. Mop up spillages immediately.

Acetic alcohol is both corrosive and highly flammable. Avoid skin contact.

Always use the fine forceps to move the root tip sample to/from solutions.

Be aware of the risk of using microscopes where direct sunlight may strike the mirror.

YOU NEED

- Garlic roots
- 1 M hydrochloric acid
- Acetic alcohol (ethanoic alcohol)
- Orcein ethanoic stain (acetic orcein)
- Ice-cold distilled water
- Water bath at 60 °C
- 2 watch glasses or small sample glasses
- Test tube
- 2 pipettes (and pipette fillers) or small measuring cylinders
- Microscope slides and coverslips
- Pair of fine forceps
- Filter paper or soft tissue paper
- Microscope with magnifications of ×100 and ×400
- Fine scissors

Procedure

1 Put a test tube containing 2 cm^3 1 M hydrochloric acid into a water bath at 60 °C.

2 Cut off about 5 mm from several root tips of some growing garlic roots using fine scissors. Choose root tips that are white and have a firm, rounded end; tips that are turning brown will give poor results.

3 Put the root tips in a watch glass containing approximately 2 cm^3 of acetic alcohol for a minimum of 10 minutes.

4 Remove the root tips and place them in a second watch glass with approximately 5 cm^3 ice-cold water. Leave for 4–5 minutes, then dry the root tips on filter paper. It is important to blot the tips well to remove the water at this stage or a precipitate may form when staining.

5 Put the root tips into the pre-heated hydrochloric acid for exactly 5 minutes.

6 Repeat step 3. Take care – the root tips will be very fragile.

7 Transfer one of the root tips to a clean microscope slide.

8 Gently break up the root tip cells with a mounted needle (this is called maceration). Add one small drop of acetic orcein stain and leave to stain for 2 minutes.

9 Cover with a coverslip, and blot firmly with several layers of tissue or filter paper. Press gently to spread the root tip, or tap gently on the coverslip with the end of a pencil.

10 View under the microscope (×400 magnification) and look for cells with visible chromosomes.

11 Look for regularly shaped, actively dividing cells. DNA stains dark red/black with acetic orcein stain so you should be able to see red/purple groups of chromosomes against a paler pink background.

12 If your preparation is not very successful, repeat with some of the other root tips from stage 6. Try to adjust your procedure to remedy the problem; for example, if your cells are over- or under-stained, adjust the quantity of stain added.

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SAFETY

Wear eye protection, lab coats and disposable gloves throughout.

Review the students' risk assessments and discuss any safety considerations.

1 M hydrochloric acid is an irritant.

Toluidine blue is harmful if ingested and will also stain skin and clothes.

Orcein ethanoic stain is corrosive, irritant, causes burns, has an irritating vapour and stains. Avoid contact with skin. If contact does occur wash the area thoroughly with water for 10 minutes. Mop up spillages immediately.

Acetic alcohol is both corrosive and highly flammable. Avoid contact with skin.

Keep stock bottles of orcein ethanoic stain and acetic alcohol in a fume cupboard.

Ensure direct sunlight cannot shine on the microscope mirrors. Demonstrate how to insert the slide correctly onto the stage. Ramming the slide may produce glass shards.

Make sure students always use the fine forceps to move the sample to/from the solutions.

Notes on the procedure

This is a Core Practical so students need to be familiar with how the stages of mitosis can be observed practically.

It is assumed that the students will be familiar with the stages of mitosis before undertaking this activity.

Two alternative practical schedules are provided. One uses toluidine blue stain. The other is the traditional one using orcein (orcein ethanoic). The technique described using toluidine blue stain is simple, cheap, safe, reliable and quick. The meristem tip is usually a denser white and is more rounded than the cut end. In the orcein ethanoic stain method, root tips may be kept for several months after step 3. Delay squashing for several hours. This allows the cells to harden, reducing the danger of them bursting. It also allows more time for them to take up the stain.

Avoid placing too much material on a slide. If cells are overlapping, squash the slide again between two wads of filter paper. *Avoid lateral movement of the coverslip.*

It has been reported that the younger the roots the better the results obtained. Roots that have just emerged produce the best results.

Notes on developing practical skills

The activity gives students the opportunity to develop practical skills, in particular microscope skills. Once the practical work is completed students could use the Developing Practical Skills Self-evaluation Sheet in Practical Skills Support 1 to reflect on the skills that have been addressed through this practical work.

The notes below relate to the numbered points in the Interpreting what you see on your cell preparation section.

1 Scientific drawing from observation with annotations is a required skill that students should have the opportunity to develop. They need to draw clearly and accurately, showing the relative sizes and positions of the chromosomes in relation to the rest of the cell. In examination questions students could be asked to annotate a diagram or drawing; they need to develop a clear understanding of what this involves. See Practical Skills Support Sheet 8 – using a microscope – for guidance on biological drawing.

2 Students need to present their results in an appropriate table. For guidance on presentation of results see the Maths and Stats Support section of SNAB Online.

3 and **4** Students do some simple calculations and then interpret their findings in relation to the process of mitosis. The length of different phases can be deduced from the number of cells present in each stage. Results will vary. The mitotic index near the tip will be higher than that further along the root because of the higher rate of division in the apical meristem. The results students obtain can be used in the interactive tutorial on mitosis counting in Activity 3.10.

5 Students make measurements using a stage micrometer and eyepiece graticule. Guidance on their use can be found in Practical Skills Support Sheet 9 – size and scale – see the support section of SNAB Online.

In the evaluation of the practical work the Student Sheet guides students to comment on a range of practical skills. The discussion about quality of results is likely to focus on the measurements made. It may include comments on how the validity of results can be ensured by carefully using an eyepiece graticule that has been calibrated correctly using a stage micrometer. Students may comment on the precision of the results and the use of the smallest divisions on the graticule scale. Comments on any repeated measurements might include that there is not a single true value so repeats will show variability and allow means to be compared. An appropriate statistical test can be completed if sufficient data is available.

Toluidine blue method adapted from SAPS Student Sheet 17 – Mitosis in root tips.
Acknowledgement: Ashby Merson-Davies, Sevenoaks School, Kent.

Orcein ethanoic stain method adapted from SAPS Student Sheet 17 – Mitosis in root tips.
Acknowledgement: Dr Kwiton Jong, Royal Botanic Garden, Edinburgh, and Rodger McAndrew, Queensferry School.

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Additional points to note

The meristem tip is usually a denser white and is more rounded than the cut end.

Avoid placing too much material on a slide. If cells are overlapping, squash the slide again between two wads of filter paper. *Avoid lateral movement of the coverslip.*

Method 1 Using toluidine blue stain

This technique is said to be simple, cheap, safe, reliable and quick.

SAFETY

1 M hydrochloric acid is an irritant. Wear eye protection, lab coats and disposable gloves.

Toluidine blue stain is harmful if ingested and will stain clothes and skin.
See website version of CLEAPSS Hazcard 32 'Dyes, indicators and stains' for further information.

Requirements per student or group of students	Notes
Root tips	*Growing garlic roots* This can be done using one of two methods: • Pushing a garlic clove into the top of a test tube of water. • Cutting small holes in a polystyrene ceiling tile and pushing the garlic cloves part-way through these holes, then floating the tile in a tray of water. Leave until roots develop (3–4 days). The best results are obtained from roots that have just emerged from the clove; roots less than 1 cm in total length are reported to work well.
1 M hydrochloric acid	
Toluidine blue stain	Toluidine blue stain can be purchased in powder form. Make up a 0.5% solution in McIlvaine buffer (0.1 M citric acid, 0.2 M sodium hydrogen phosphate at pH 4). This should keep for many months at room temperature.
Cold distilled water	
2 watch glasses or small sample tubes	
Hollow glass block or small sample tube	
2 pipettes (and fillers) or small measuring cylinders	To measure 2 and 5 cm^3.
Microscope slides and coverslips	All slides and coverslips should be cleaned with alcohol.
Pair of fine forceps	
Filter paper or soft tissue paper	
Microscope with magnifications of ×100 and ×400	
Eye protection	

Method 2 Using orcein ethanoic stain

SAFETY

Wear eye protection, lab coats and disposable gloves.

1 M hydrochloric acid is an irritant.

Ethanoic acid is corrosive and very irritating to the respiratory system. Industrial alcohol is toxic and highly flammable. Carry out preparation in a fume cupboard.

The stock solution should be labelled 'CORROSIVE' and kept within the fume cupboard. See website version of CLEAPSS Hazcard 32 'Dyes, indicators and stains' for further information.

Set up microscopes in an area without direct sunlight.

Requirements per student or group of students	Notes
Root tips	*Growing garlic roots* This can be done using one of two methods: ● Pushing a garlic clove into the top of a test tube of water. ● Cutting small holes in a polystyrene ceiling tile and pushing the garlic cloves part-way through these holes, then floating the tile in a tray of water. Leave until roots develop (3–4 days). The best results are obtained from roots that have just emerged from the clove; roots less than 1 cm in total length are reported to work well.
1 M hydrochloric acid	
About 2 cm^3 of acetic alcohol	3 parts of absolute alcohol: 1 part of glacial ethanoic (acetic) acid, which should be freshly mixed. Industrial spirit or 95% ethanol can be used in place of absolute alcohol, but chromosomes may not be as clearly defined. Root tips may be kept for several months after step 3. Delaying squashing for several hours allows the cells to harden, reducing the danger of them bursting.
A small drop of orcein ethanoic stain	Lacto-propionic orcein or acetic orcein can be used. Concentrated lacto-propionic orcein stain is diluted to a 45% solution by volume with distilled water. The stain must be filtered prior to each use. Orcein ethanoic stock solution contains 2.2 g orcein dissolved in 100 cm^3 glacial ethanoic acid. Dilute 10 cm^3 of stock solution with 12 cm^3 of water before use. The diluted solution does not keep.
5 cm^3 of ice-cold distilled water	
Water bath at 60 °C	
2 watch glasses or small sample tubes	
Test tube	
2 pipettes (and fillers) or small measuring cylinders	To measure 2 and 5 cm^3.
Microscope slides and coverslips	All slides and coverslips should be cleaned with alcohol.
Pair of fine forceps	
Filter paper or soft tissue paper	
Microscope with magnifications of ×100 and ×400	
Eye protection	