Scientific drawing of cells from slides of root tip to show stages of mitosis

Specification reference: **1.2**

Genetic material is copied and passed on to daughter cells

Introduction

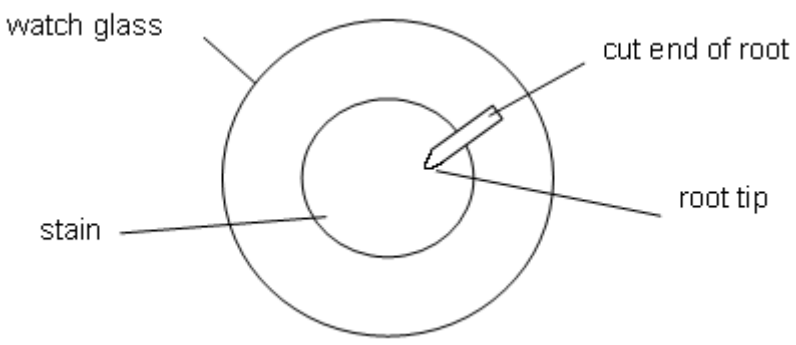
Mitosis is a process of cell replication needed for growth and repair. Onion (*Allium* sp.) is very useful for root tip preparation to study the different stages in mitosis. At the tip of the root there is an apical meristem where cells divide by mitosis. To observe the stages of mitosis the root tissues must be well fragmented. Hydrochloric acid is used to separate the cells by breaking down the tissue which binds cells together (maceration).

Apparatus and reagents

Microscope	Bunsen burner / hot plate
Garlic or onion with developing roots	Microscope slide and cover slit
Scalpel	Fine forceps
Watch glass	Mounted needle
acetic-orcein stain	Dropping pipette
1 M hydrochloric acid	Paper towel

Method

1. Cut 10 mm from the tip of a growing root.
2. Place 20 drops of acetic-orcein stain and 2 drops of 1 M hydrochloric acid into a watch glass.
3. Place the root tip so that the tip is in the stain and the cut end facing the outside of the watch glass as shown in the diagram.



4. Remove 2–3 mm from the tip of the root and place it on a microscope slide.
5. Add 2–3 drops of acetic-orcein stain.
6. Warm the slide under gentle heat for 4–5 seconds.
7. Completely break up the tissue with a mounted needle.
8. Apply a cover slip, place the slide and coverslip on a layer of paper towel and fold the paper towel over the coverslip. Make sure that the slide is on a flat surface and squash down on the coverslip with a strong vertical pressure using your thumb. Do not twist or roll the thumb from side to side.
9. Use a microscope with the x 10 objective to locate the zone of cell division (apical meristem). The cells in this region are 'square' in shape with nuclei which are large relative to the whole cell area. If you see xylem vessels with their characteristic spiral thickening you are looking in the wrong area ....not at the tip!
10. If the cells are overlapping, squash again.
11. Using the x 40 objective lens, observe and draw, cells at interphase, prophase, metaphase, anaphase and telophase.

Risk assessment

Hazard	Risk	Control measure
Ethanoic ethanol is corrosive	Could be transferred to skin or eyes during experiment	Wear gloves/safety glasses
Propionic-orcein stain contains propionic acid and is corrosive	Could be transferred to skin or eyes during experiment	Wear gloves/safety glasses
Hydrochloric acid is corrosive	Could be transferred to skin or eyes during experiment	Wear gloves/safety glasses
Scalpels are sharp	Could cut skin when cutting roots	Cut away from body onto white tile

Teacher/ Technicians notes

Use shallots or small onions. Garlic is often suggested because fresh cloves will sprout very quickly.

Support the onion or garlic clove so that it just touches the surface of water. It can be supported by sticking a cocktail stick through the onion or clove of garlic and supporting on the neck of a wider vessel. Alternatively cut holes in a polystyrene sheet insert the cloves and float on the surface of water.

Roots that have been set to sprout for 2–5 days seem to give the highest likelihood of finding actively dividing cells.

Some people find that cutting the root tips close to midday makes a difference to the number of dividing cells so you may want to cut and fix root tips at this time and give these to the students. They can be fixed in ethanoic alcohol and kept in this fluid for several months. Ethanoic alcohol is 3 parts absolute alcohol to 1 part glacial ethanoic acid. Mix just before use by adding the acid to the alcohol.

Propionic orcein stain

Grind 1.5 g of solid orcein with a pestle and mortar.
In a fume chamber mix 90cm^3 of glacial propionic acid with 110 cm^3 of distilled water and bring to the boil.
Pour the boiling mixture over the orcein and stir thoroughly.
Leave overnight and then filter and store in a lightly stoppered dark bottle.
If the chromosomes are overstained dilute the stain with 45% propionic acid.
Ethanoic acid can be used rather than propionic acid but propionic acid has the advantage that it evaporates more slowly.
More information is available from:

http://www.nuffieldfoundation.org/practical-biology/investigating-mitosis-allium-root-tip-squash

It is acceptable to use prepared slides of root tip if you so wish.

Further work

- Alternative to onion or garlic could be used for example seedlings of beans (2n 22) or peas (2n 14).
- Students could calculate the mitotic index:

  $$\text{Mitotic index} = \frac{\text{number of actively dividing cells in field of view}}{\text{Total number of cells in field of view.}}$$

- The effect of variables such as time, temperature, light on the mitotic index could be studied.

Practical skills

- use laboratory glassware apparatus for a variety of experimental techniques to include serial dilutions
- use qualitative reagents to identify biological molecules
- produce scientific drawing from observation with annotations
- use of light microscope at high power and low power, including use of a graticule