

Mitosis in growing root tips

Cells in actively growing tissue go through a cycle of metabolic activity, DNA replication, chromosome segregation and cell division known as the cell cycle. Mitosis, the process of chromosome condensation and separation into new daughter nuclei, is one part of the cell cycle. The characteristic changes in the appearance of chromosomes as they proceed through the stages of mitosis, prophase, metaphase, anaphase and telophase, can be identified by staining the chromosomes.

This procedure describes the staining of chromosomes in growing root tips of garlic, which has nice compact cells that usually give good staining (and consistently better results than is obtained with onion root tips). Root tips are grown and preserved in acetic ethanol fixative. Fixed root tips can be stored for at least two weeks prior to staining. Treatment with acid and heat is used to break up the cellulose cell wall allowing stain to permeate the tissue and makes it easier to squash the tissue on a microscope slide. Aceto-orcein stain turns chromosomes a purple-red colour. This stain can be prepared from powder or purchased as solution; staining is best with freshly prepared stain as, over time, the stain precipitates and changes colour from a deep red or burgundy colour to a brownish colour.

Materials needed for each student pair

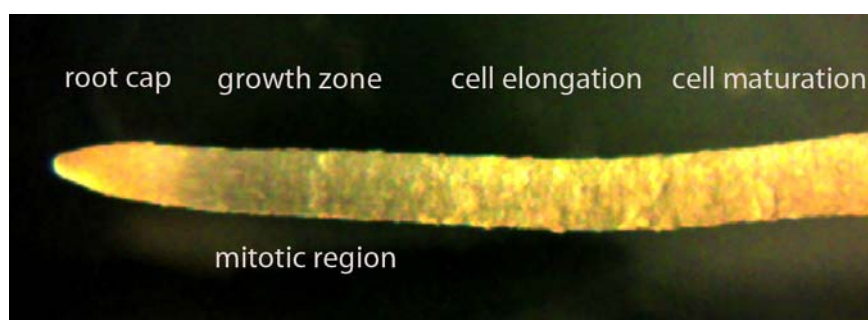
- | | |
|--|---|
| <input type="checkbox"/> Garlic root tips (<i>Allium sativum</i> , $2n=16$) fixed in Acetic ethanol fixative (3:1 ethanol:acetic acid) | <input type="checkbox"/> Spare growing garlic roots (to demonstrate the origin of the roots and as backups) |
| <input type="checkbox"/> 1M hydrochloric acid, 1mL | <input type="checkbox"/> 2 Microscope slides and cover slips |
| <input type="checkbox"/> Aceto-orcein stain, 0.5mL
OR 0.05% Toluidine blue | <input type="checkbox"/> Razor blades or scalpels |
| <input type="checkbox"/> 60°C oven, hot plate or Bunsen/spirit burner | <input type="checkbox"/> Forceps |
| <input type="checkbox"/> Glass rod with smooth rounded end | <input type="checkbox"/> 2 transfer or Pasteur pipettes |
| <input type="checkbox"/> Compound microscope with 400x magnification and 1000x oil immersion | <input type="checkbox"/> Safety glasses |

Growing garlic root tips

Suspend garlic cloves over water, with the base where roots grow just in the water. Leave in the dark for 7-10 days. Periodically check that the base is still in water. Grow the roots until they are about 2-3 cm long. The mitotic region of the root is a little behind the root cap.



For fixation, cut the 1.5-2 cm end of the root and place directly into fixative.



Sourcing Materials

Aceto-orcein stain	Solution from Southern Biological Product SI1 Aceto-orcein, 1%, 25mL (\$36.50 in Dec 2012). Do not use the stain if it has lost its rich burgundy colour. Use this stain as it is, undiluted. Aceto orcein powder can be purchased and the solution prepared according to the procedure below. <i>Note: stains like this one can be messy and difficult to prepare, may need to be prepared in a fume hood and require lengthy heating to dissolve. And there is a good chance of contaminating the whole lab with the stain. So best to purchase already prepared.</i>
Toluidine Blue, 0.05% solution	Southern Biological SI18 - Toluidine blue, 0.05% available in 100mL & 500mL Science Supply Australia 3268/10G Toluidine Blue (C.I.52040) \$74.95 (ex GST)
Ethanol	Standard school lab chemical suppliers e.g. Haines
Acetic acid	Standard school lab chemical suppliers e.g. Haines
Hydrochloric acid	Standard school lab chemical suppliers e.g. Haines

Preparing acetic ethanol fixative

Mix 3 parts of absolute ethanol (ethyl alcohol) with 1 part glacial acetic acid
e.g. for 40mL fixative: 30mL absolute ethanol + 10mL glacial acetic acid

Fixing the root tips

Grow the roots until they are 2-3 cm long
Cut the 1.5-2 cm end off the root. Place immediately into acetic ethanol fixative
Fix for at least 2h before staining.
Store at 4°C

Aceto orcein stain

Aceto-orcein stain can be purchased as a 1% solution from Southern Biological. Use this stain undiluted.

Preparing aceto-orcein stain from powder (the harder option)

Stock solution: 2g synthetic orcein + 45 mL glacial acetic acid.

Dissolve the orcein in gently boiling glacial acetic acid. This takes considerable time so do this in a conical flask with a filter funnel placed in the neck to act as a simple condenser, and do it in a fume hood. Cool and filter. Store in the dark.

Working solution: Add 10mL stock solution to 12 mL distilled water.

Place in dropper bottles for easy application. Dark glass dropper bottles are best.

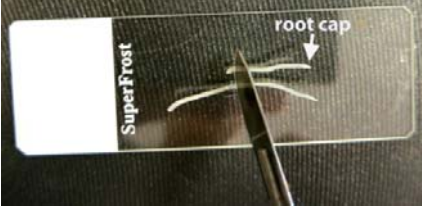
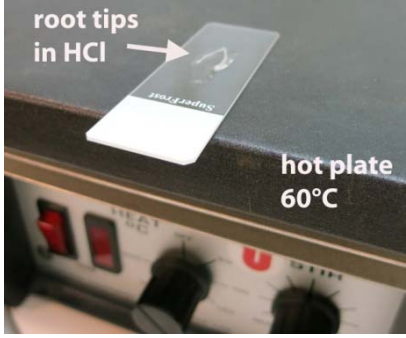
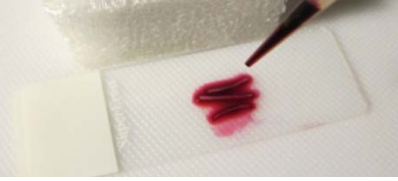

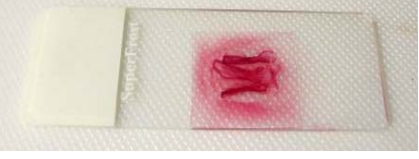
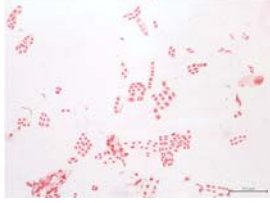
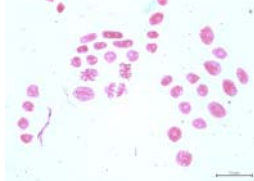
Alternative stain – Toluidine blue 0.05% - use at this concentration. This stain is classified non-hazardous.

Safety and MSDS

1. Wear safety glasses
2. MSDS required for
 - Aceto-orcein stain
 - 1M HCl (dilute hydrochloric acid)
 - Acetic acid (glacial)
 - Absolute Ethanol (ethyl alcohol)

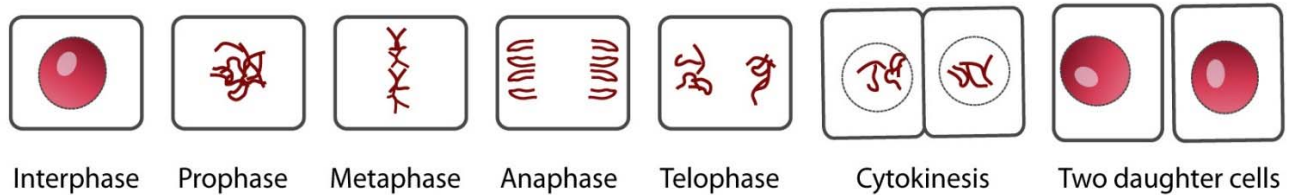
Method: Staining and squash preparation

Wear safety glasses

1	The root tips have been grown and placed into fixative solution prior to class (min. 2h fixation).	
2	Remove 2-3 roots from the fixative with forceps. If necessary, use a razor blade cut off the 1-1.5 cm end portion (the pointy growing root tip) and discard the rest [make sure you choose the growing end of the root, not the portion that was closer to the garlic clove]. Place the root tips on a glass slide with 4-5 drops of 1M HCl (enough to cover them).	
3	Place the slide with root tips in acid at 60°C for 2 min (e.g. 60°C oven or hot plate or heat by holding about 30cm above a Bunsen burner flame with a peg - 60°C is just tolerable to touch but protect your fingers from the heat by using a safety peg)[it is important that the solution does not boil or dry out; add more HCl if it is drying].	
4	Add 2 drops of aceto-orcein stain (or Toluidine blue stain) to the root tips. Sit for 1min to allow the stain to penetrate the tissue. <i>(you may need to remove excess HCl or transfer the root tips to a new slide)</i>	
5	Place a coverslip over the roots. Fold a small piece of tissue paper to about the size of the coverslip, then, with the tissue paper over the coverslip, gently and evenly press down with a finger or thumb to squash the roots.	 
6	View under low power to see if you have cells from the actively growing area of the root tip. The dividing garlic root cells are usually cuboidal (suarish in shape) with many large obvious nuclei. If the cells are elongated (long rectangles) you have cells from too far back in the root that are no longer dividing.	
7	View under high power 400x 1000x oil immersion if possible	
8	Draw or photograph cells at different stages of mitosis.	

In actively growing root tips you usually find at least a few cells at various stages of mitosis. The majority of cells will be in interphase, so it is important to go to 400x magnification to clearly see the mitotic cells. It takes a bit of time viewing the cells carefully to start to recognise the different stages. Use the diagram below to help identify the stages of mitosis.

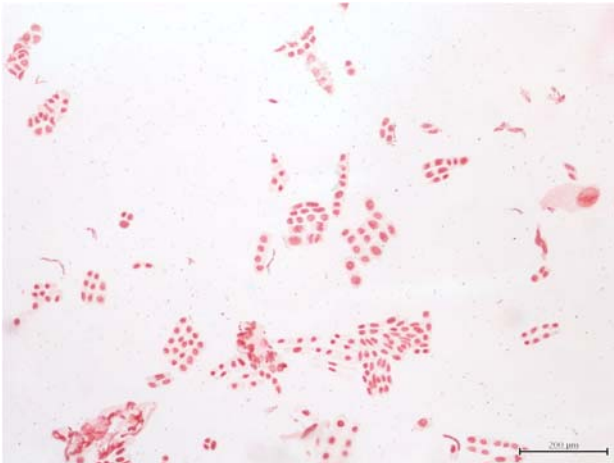
Stages of mitosis



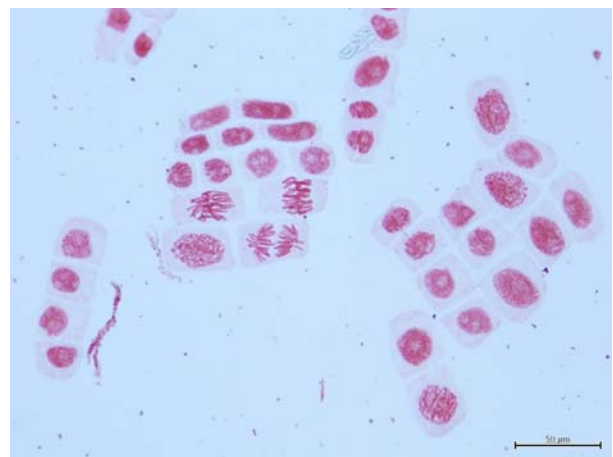
Photomicrographs: results from GTAC staining procedures on garlic root tips

Aceto-orcein

100x magnification

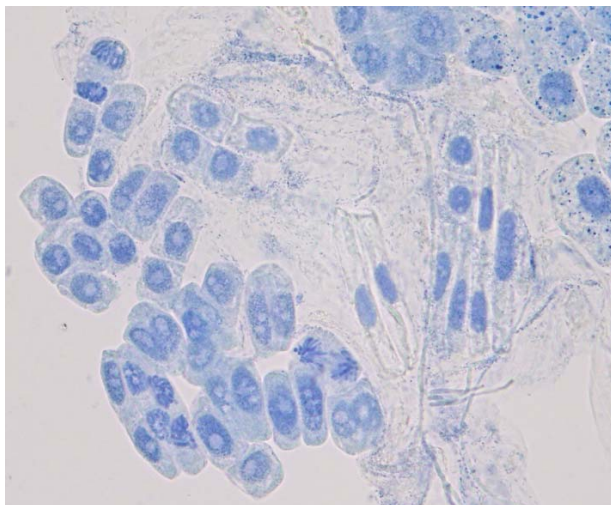


400x magnification



Toluidine blue

400x



1000x

