S4 - S6 Senior Phase

# "Divide"

## Observing mitosis and determining the mitotic index

**N5 Biology, Unit 2, KA1:** Producing new cells. **Higher Biology, Unit 1, KA1**: Division and differentiation in human cells. **AH Biology, Unit 1, KA5**: Protein control of cell division.

Cell division by mitosis can be observed by microscopy within meristemic tissue of plant roots and shoots.

### Step 1: Preparing the slide.

Materials

- Garlic roots grown between 2-4 days.
- 1M hydrochloric acid
- Toluidine blue stain
- Watch glass
- Microfuge tube

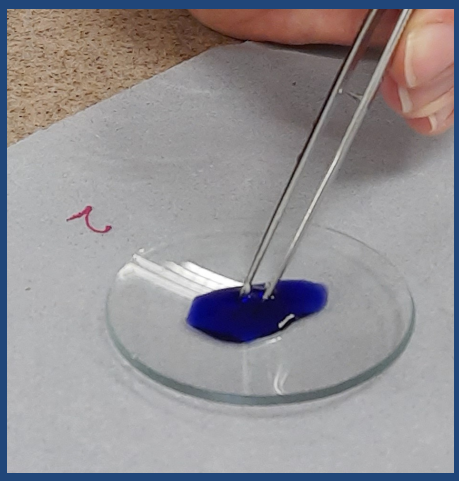

- Dropping pipette
- Paper towel
- Light microscope, slide and coverslip
- Fine scissors + forceps
- Water bath set to 50°C.
- Distilled water

- 1M hydrochloric acid is an irritant - wear eye protection, lab coat and disposable gloves.
- Toluidine blue is harmful if ingested. It will stain skin and clothes but is less hazardous than acetic orcein, which is traditionally used in this practical.

**1** Gently wedge a clove of garlic in the neck of a boiling tube or test tube (or an ice-cube tray as we did here). The garlic was left in water for **2-4 days** to allow roots to grow.

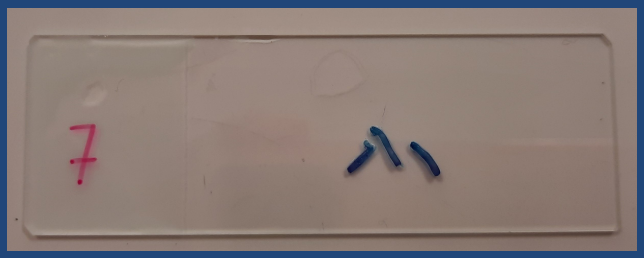

**2** Using scissors, cut the terminal 3mm off the garlic roots. Place them in a microfuge tube containing 1M hydrochloric acid. Place in a water-bath at 50°C for 5min.

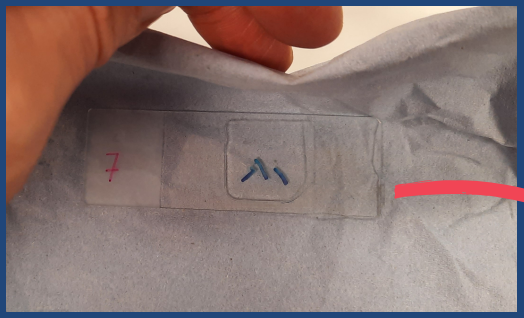
3 Use a dropping pipette to remove the acid from the microfuge tube. Wash several times with distilled water.

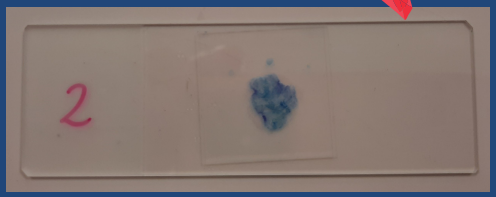


4 Use forceps to place the root tips on a watch-glass. Flood the tips with toludine blue for 2 minutes. Once the stain time has elapsed, remove the excess stain from the watch glass and thoroughly rinse with distilled water. Transfer a couple of root tips to a glass microscope slide (less is more we found!).

5 Use a pipette to add a small drop of water to the microscope slide and then cover with a coverslip.

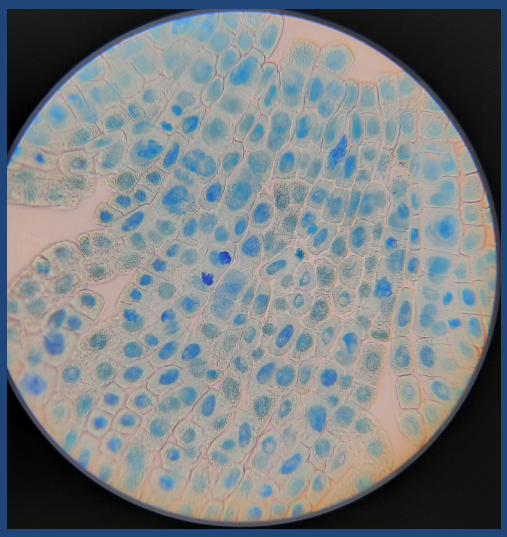






6 Wrap the slide in a paper towel and gently press down on the coverslip with a thumb to squash the root tips. Be very careful not to slide the coverslip.

7 View the root tip squash under a compound light microscope using increasing magnification. Scan the preparation for evidence of mitosis.

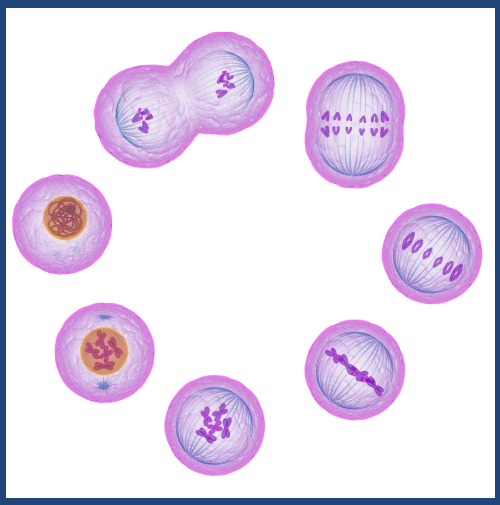


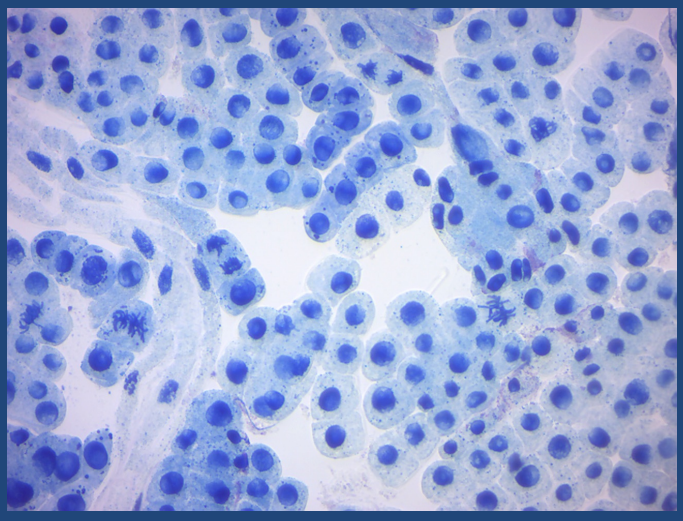
## Step 2: Determining the mitotic index

Materials

- Light microscope
- Slide prepared in Step 1

1 Identify cells in each stage of mitosis: interphase, prophase, metaphase, anaphase and telophase.



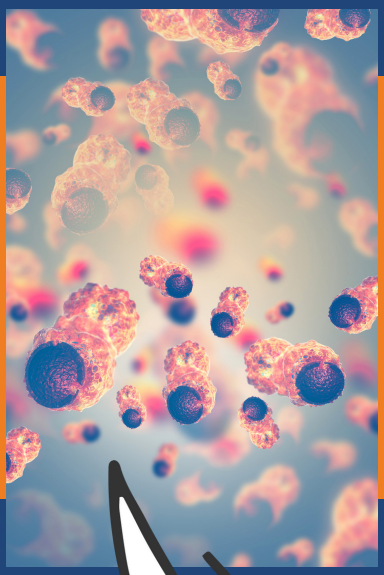


2 Count the number of cells in the area visible under the microscope when viewed at x400 magnification. Count the number of cells in each stage of mitosis.

3

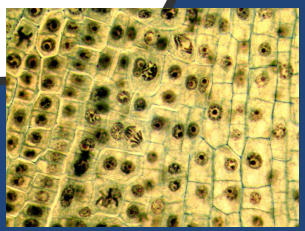
The amount of cell division occurring in a tissue can be quantified using the mitotic index. Use the formula below to calculate the mitotic index for your root tip.

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



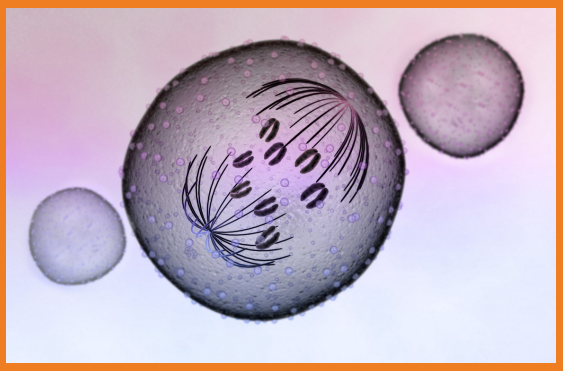
The mitotic index is useful in studies of cell division in many different types of tissues, e.g. in examining tumour growth in cancer patients. In this case, it can shed light on the frequency of cell division within a tumour and, therefore, how quickly the tumour is growing.

**Push Yourself Further**: There are many interesting alternatives to garlic. Why not try the same experiment using hyacinth bulbs, onions and broad beans [1].



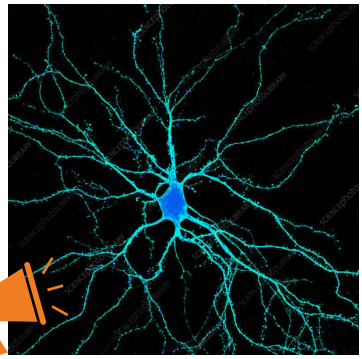
## References

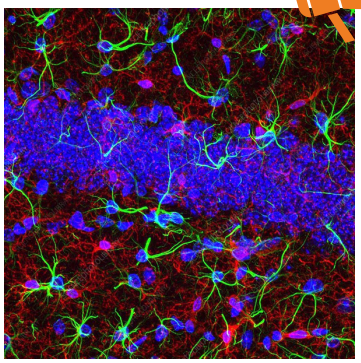

[1] SAPS, "A-level set practicals: Microscopy of root tip mitosis", web-link here. The protocol published here was based, with modifications, on this one published by SAPS. The modifications particularly relate to the time recommended to let the garlic roots grow. Minimal evidence of mitosis was observed if the roots were left to grow for more than 5 days.

# Microscopes in the world of work

Dr Chris Henstridge, a principal investigator within the School of Medicine in Dundee uses microscopes to learn about neurological conditions.





We asked Chris the two questions below. Click on the orange loudspeakers to hear his reply. Click on the "transcript" button below for the full conversation.

What do you think is important to be a scientist?

What are you trying to find out in your lab?

Transcript