## NEATH PORT TALBOT COLLEGE COLEG CASTELL NEDD PORT TALBOT

## School of Maths & Science Science Practical

# Mitosis in Hyacinth / Garlic root tip cells using Toluidine Blue stain

#### **♦** Aim

To see chromosomes and to observe stages in mitosis in plant cells.

#### **◆** Introduction

If the chromosomes are selectively stained with a dye such as Toluidine blue, stages in mitosis can be observed in tissues where cell division occurs. An example of such a tissue is the meristematic tissue located in the zone of cell division, in the tip of a growing root.

## **♦** Safety



#### **Control Measures**

- The wearing of safety glasses and a laboratory coat at all times will be sufficient to take account of most hazards and significant risks.
- Avoid contact with Toluidine blue stain and hydrochloric acid.
- You are reminded of the need for good laboratory practice in order to maintain a safe working environment.



Hazards

**Irritant** 

2 mol / dm<sup>3</sup> Hydrochloric acid Toluidine Blue stain

#### **♦** Procedure

- 1. Place a few drops of de-ionised water in the watch glass labelled  $H_2O$ .
- **2.** Place a few drops of 2 mol / dm<sup>3</sup> Hydrochloric acid in the watch glass labelled HCl.
- **3.** Using fine forceps, remove an intact root from the rooting hyacinth / garlic, and lay it on a microscope slide. Use a scalpel to cut off approximately 0.5cm of the extreme tip of the root.
- **4.** Place the tip of the root in the watch glass containing the Hydrochloric acid and leave it there for 3 minutes.
- 5. When 3 minutes is up, transfer the root tip to the watch glass containing the deionised water, gently move the root tip around in the water to rinse off any acid and then gently transfer it to a clean microscope slide.
- **6.** Remove any water from the slide by using a corner of tissue to soak up the water by capillary action.
- 7. Add one drop of Toluidine blue stain to the root tip.
- **8.** Place a cover slip over the stain and tap it carefully with the forceps. You should see the root tip cells separate out but the cells in the middle will be white and unstained.
- **9.** Carefully push the coverslip so that a corner projects over the edge of the slide. Lift the corner slightly with the forceps so that the stain washes over all the cells. Do this two or three times.
- **10.** Reposition the coverslip in the middle of the slide and tap it again with the forceps. You should see the cells well spread out and a medium blue colour.
- 11. Place the slide on some tissue paper, fold the tissue over the top and press gently to absorb any excess stain. There may be a lot of air bubbles this is normal.
- **12.** Now examine your preparation. Ideally you will see dark blue nuclei and almost colourless cytoplasm. Identify and draw cells in interphase and in different stages of mitosis.
- **13.** Examine a prepared slide of Hyacinth / Garlic root tip and draw a plan diagram to indicate the zone of cell division i.e. where the stages of mitosis can be seen.