

LOOKING AT PLANT STEMS

Purpose

- To look at the structure of xylem vessels, phloem sieve tubes and sclerenchyma fibres.
- To locate the position of these tissues within the stem.
- To develop practical skills including microscope use, biological drawing and measuring using an eyepiece graticule.

Part A: Looking at tissues

The procedure below lets you look at the structure of vascular tissue in rhubarb.

SAFETY

Wear eye protection, lab coats and disposable gloves.

Avoid contact with methylene blue as it stains clothes and skin. See CLEAPSS Hazcard 32 for further details.

If liquid comes in contact with skin, flood area of skin with water and then wash thoroughly with soap and water.

Be aware of the danger of using microscopes where direct sunlight may strike the mirror. Take care to avoid cuts caused by broken coverslips.



YOU NEED

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| <ul style="list-style-type: none"> • Small piece of tinned rhubarb • Microscope slide • Coverslip • 2 mounted needles • Forceps | <ul style="list-style-type: none"> • Watch glass • Methylene blue (1% solution) • 50% glycerol • Filter paper |
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Procedure

- 1 Place a small piece of tinned rhubarb on a watch glass. Use forceps to pick out one or two vascular bundles from this block of tissue and place them on a microscope slide.
- 2 Use mounted needles to tease the vascular bundles apart. Cover the tissue with a drop of methylene blue, and leave for 5 minutes.
- 3 Draw off the extra stain with filter paper. Place a drop of dilute glycerol on the fibres and mount under a coverslip.
- 4 Examine your preparation under low, medium and high magnification. If the tissues are not separated enough, place your slide on a piece of filter paper, put a filter paper pad on the coverslip and press down with your thumb. This may separate out the tissue. Do not move your coverslip sideways at all. You may need to re-irrigate the slide with glycerol after squashing it. To do this, place a drop of glycerol on the slide next to the coverslip. It will be drawn under the coverslip by capillary action. Blot off any excess and re-examine the slide.
- 5 Look for vascular bundles amongst the separated tissues. Use Figures 4.43 and 4.44 in the Student Book (pages 181 and 182) to help you identify the different tissues.
 - The xylem vessels are empty, elongated tube-like cells; they may show different types of wall thickening, for example, spiral or annular (in rings) thickening or, in some cases, virtually complete lignification of the walls.
 - Phloem sieve tube elements are also elongated; they lack a nucleus even though the cells are alive with some cell cytoplasm. There are sieve plates with pores between adjacent cells. Each sieve tube element is associated with a companion cell.

Make drawings of the different types of cells you can identify.

Part B: Exactly where are the ‘fibres’ found?

You can either make your own sections of a plant stem or look at ready prepared slides. The procedure given below is for cutting hand sections of buttercup or any herbaceous stem.

SAFETY

Wear eye protection, lab coats and disposable gloves. Avoid inhalation and skin contact.

Acidified phloroglucinol is corrosive and highly flammable. See CLEAPSS Hazcard 12 for further details.

If the liquid comes in contact with skin, flood the area of skin with cold water for 10 minutes and then wash thoroughly with soap and cold water.

Take care when using a scalpel or razor blade.

Be aware of the danger of using microscopes where direct sunlight may strike the mirror. Take care to avoid cuts caused by broken coverslips.



YOU NEED

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| <ul style="list-style-type: none"> • Piece of stem from herbaceous plant • Acidified phloroglucinol (benzene-1,3,5-triol) in ethanol with concentrated hydrochloric acid • Sharp scalpel • New razor blade • Watch glass | <ul style="list-style-type: none"> • Paintbrush • Pipette • Microscope • Microscope slide • Coverslip • Wax crayon |
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Procedure

- 1 Use a sharp scalpel to cut out a piece of stem.
- 2 Hold the stem as shown in Figure 1 and cut thin transverse sections across it using a moistened new razor blade.

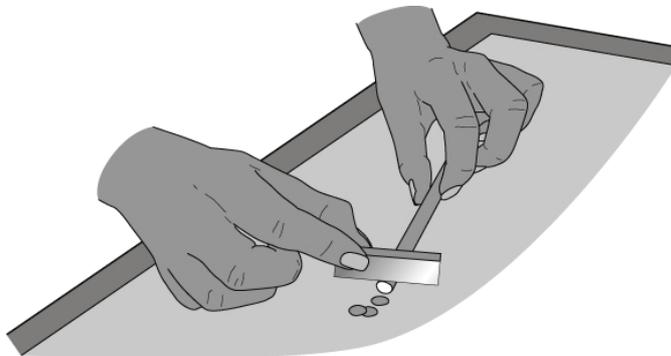


Figure 1 Cutting thin transverse sections of a stem using a razor blade.

- 3 Cut a lot of sections. You do not need a complete section across the stem, as a small segment will be sufficient. Use a paintbrush to transfer your sections to a watch glass of water.
- 4 Select the thinnest sections and transfer to a slide. Using a wax crayon, draw a line from top to bottom of the slide on both sides of the specimen to stop the dye spreading.
- 5 Add a few drops of acidified phloroglucinol and a coverslip.
- 6 Examine under a microscope.
- 7 Use your sections and/or a prepared slide of a cross-section across a dicotyledonous stem, such as *Helianthus* or a member of the Cucurbitaceae family, to draw a low-power plan drawing. Identify and label the position of the vascular bundles (xylem, cambium, phloem and any sclerenchyma).
- 8 Use an eyepiece graticule and stage micrometer to measure and compare the mean diameter of the xylem vessels and phloem sieve tubes. See Practical Skills Support Sheet 9 – size and scale – for information on the use of a stage micrometer and eyepiece graticule.

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SAFETY

Ensure eye protection, lab coats and disposable gloves are worn throughout.

Acidified phloroglucinol is corrosive and highly flammable – avoid inhalation and skin contact. See CLEAPSS Hazcard 12 for further details.

Avoid contact with methylene blue as it stains clothes and skin. See CLEAPSS Hazcard 32 for further details.

If liquid comes into contact with skin, flood the area of skin with water and then wash thoroughly with soap and water.

Students must take care when using scalpels or razor blades. Demonstrate a safe method for cutting materials.

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

Demonstrate how to insert the slide correctly onto the stage. Ramming the slide may produce glass shards. Take care to avoid cuts caused by broken coverslips.



Notes on the procedure

It is possible to use any plant stem for Part A if soaked in macerating fluid; details can be found in CLEAPSS. However the tinned rhubarb is much simpler, safer and produces good results.

Ready prepared slides can be used for viewing sections of stems rather than students cutting their own.

The Core Practical requirement is the identification of xylem vessels, sclerenchyma fibres and phloem sieve tubes and their location within stems through a light microscope. If time permits, students could look at the route of xylem vessels through stems. A procedure for this practical work is included on the next page.

The dissection of the broad bean stems takes patience and a light touch. When completed it shows how vessels divide, with one part then going into the leaf petioles and another part continuing up the plant.

Notes on developing practical skills

In this Core Practical the focus is on the use of light microscopy at low and high power, including use of a graticule, and scientific drawing from observation with annotations. See Practical Skills Support Sheet 8 – using a microscope, and Sheet 9 – size and scale, for information on the use of a stage micrometer and eyepiece graticule.

The practical method using rhubarb for this activity is based on one supplied by Janice Green, Esher College, Surrey.

Part C: Route of xylem vessels through the stem

A cross-section of the stem gives a 2D image of where the vascular tissue is located within the stem. To get a 3D picture you either have to cut a series of longitudinal sections of the stem or dissect out the stained vessels. Both of these procedures can conveniently be done with broad bean plants.

SAFETY

Take care when using a scalpel or razor blade. Demonstrate a safe method for cutting materials.

YOU NEED

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| <ul style="list-style-type: none">• Broad bean plant about 15–20 cm tall with several leaves• Beaker of dye, for example, eosin | <ul style="list-style-type: none">• Sharp scalpel or new razor blade• Hand lens |
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Procedure

- 1 Cut a broad bean plant just above soil level.
- 2 Place the cut end in a beaker of dye for approximately 30 minutes.
- 3 With a great deal of care, scrape away the outer layers of tissue from the stem to reveal the xylem vessels that have taken up the dye. Careful dissection of the stem should reveal the route that the xylem vessels take up the plant and into the leaves.

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SAFETY

Wear eye protection, lab coats and disposable gloves.

Avoid contact with methylene blue as it stains clothes and skin. See CLEAPSS Hazcard 32 for further details.

Acidified phloroglucinol is harmful by inhalation, contact and if swallowed. It is corrosive and highly flammable. See CLEAPSS Hazcard 12 for further details.

If liquid comes in contact with skin, flood area of skin with water and then wash thoroughly with soap and water.

Do not place daylight illumination microscopes on benches that will catch the direct rays of the Sun.



Part A: Looking at tissues

Requirements per student or group of students	Notes
A small piece of tinned rhubarb	
A few drops of 1% methylene blue solution	
A few drops of 50% glycerol	
Microscope	
Microscope slide	
Coverslip	
2 mounted needles	
Fine forceps	
Watch glass	
Filter paper	
Eye protection	

Part B: Exactly where are the 'fibres' found?

Requirements per student or group of students	Notes
Prepared slide of a cross-section across a dicotyledonous stem	For example, T.S. <i>Helianthus</i> stem. The equipment below is not required if prepared slides are used.
Piece of stem from a herbaceous plant	
Acidified phloroglucinol	
Sharp scalpel	Scalpel blades should be new or very clean. If blades are not cleaned immediately after use, they should be disposed of in a secure container.
New razor blade	Safety razor blades should be used.
Watch glass	
Paintbrush	
Pipette	
Microscope	
Microscope slide	
Coverslip	
Wax crayon	To draw lines on the slide to stop the dye spreading.
Eye protection	

Safety checked, but not trialled by CLEAPSS. Users may need to adapt the risk assessment information to local circumstances.

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Part C: Route of xylem vessels through the stem

Requirements per student or group of students	Notes
Broad bean plant about 15–20 cm tall with several leaves	In the early summer these can be bought from garden centres, or grown from seed.
Beaker of dye	For example, eosin.
Sharp scalpel	
New razor blade	Safety razor blades should be used.
Hand lens	

Notes