Core practical 2: Use a light microscope to observe and measure biological samples

Objectives

- Be competent in the use of a microscope at high and low power, including the use of a graticule (eyepiece micrometer) to make measurements
- Know how to record observations using appropriate biological drawings
- Understand the importance of staining specimens in microscopy

Safety

- Toluidine blue O stain is harmful if swallowed but is not otherwise classified as hazardous. However, there is always the possibility of unknown effects and the stain should therefore be used with caution.
- Skin contact with toluidine blue O stain should be avoided.
- Wear gloves and eye protection when handling stain.
- Clean up any spills immediately and dispose of excess stain in the receptacle provided.
- To avoid cuts, take great care when using and carrying razor blades. Do not leave them lying on the desk. Your teacher will collect used blades and dispose of them in a sharps box.
- Ensure students understand the safe use of a microscope: warn against being vigorous with slides as they can splinter and advise on safe use of mounting needles.

Specification links

- Practical techniques 4, 5
- CPAC 1a, 4a

Procedure

1. Before starting this core practical, familiarise yourself with the parts of the microscope and how to focus. Check that the lens and eyepiece are clean, using lens tissue to clean them if necessary as normal tissues or cloth can scratch lenses. If present, the condenser will need to be adjusted for each magnification. To correctly focus the condenser, place a slide on the stage and a pencil point on the light source, and adjust the condenser until both are in focus. If you use a mirror for illumination, take care not to use direct sunlight, as this can cause severe damage to eyes. Instead, use a bench-lamp where possible.
2. Calibrate the eyepiece graticule (see **fig A** in the Student sheet). Place a micrometer slide on the stage of the microscope. Using the low-power objective, focus on the micrometer scale. The smallest division of the micrometer scale is usually 100 µm. Move the slide and rotate the eyepiece to align the scales of the eyepiece graticule and the stage micrometer in the field of view. Count the number of divisions (eyepiece units or epu.) on the eyepiece graticule that are equivalent to a known length on the micrometer slide and work out the length of each eyepiece unit. For example, if 100 µm is equivalent to 4 epu, then each epu is $\frac{100}{4} = 25\,\mu m$ at this magnification. Repeat this for the medium- and high-power objectives.
3. Collect a piece of plant stem. Add a few drops of water to the centre of the white tile and wet the razor to reduce friction. Hold the plant stem firmly, keeping your fingers away from the edge of the razor. Cut several transverse sections, keeping them as thin as possible. Incomplete thin sections may sometimes be better than thicker complete ones. Use a brush to transfer the sections to water in a watch glass.
4. Select the two thinnest sections and place them on separate slides. Mount one in a drop of water, lowering a coverslip onto the section using the mounted needle and making sure there are no air bubbles.

5. For the second stem section, remove excess water by carefully touching the edge with absorbent paper. Wearing glove and eye protection, add two drops of toluidine blue O stain and leave for 2–4 minutes. Add the coverslip as before and gently remove excess stain with a paper towel. Toluidine blue O is a metachromatic stain, meaning that it reacts with different chemical components of cells to produce a variety of colours. This can provide information on the nature of the cell. Toluidine blue O stains lignin and tannins green to blue, pectins pinkish-purple, and nucleic acids purplish or greenish blue.
6. Turn the objective lens to low power. Examine first the unstained and then the stained slides under the microscope. To do this, bring the lens as close to the slide as possible while watching it side-on. Then, looking through the eyepiece, focus using the coarse focusing knob, moving the lens away from the stage. This avoids damage to the slide and lens. Use the fine focus until a clear view of the section is established. Compare what can be seen in the stained and unstained slides.
7. For the stained slide, remain on low power and draw and annotate a simple outline plan of the section. Show the arrangement of tissues within the stem but do not include any cell details (see **fig B** in the Student sheet).
8. Using the eyepiece graticule, measure a vascular bundle and/or the stem diameter. Add a scale bar to your diagram. Add a title and include the magnification at which you made your observations. For example, with an eyepiece lens magnification of ×10 and an objective of ×10 the total magnification will be ×100. Remember this is not the same as the magnification of the drawing.
9. Now turn the objective disc to the medium power lens, again focusing until the cells are clear and distinct. Finally, turn the objective disc to high power lens and focus with the fine focusing knob only. Choose no more than 10 cells at the junction of two tissue types. Draw and label the detail of these cells as accurately as you can (see **fig B** in the Student sheet).
10. Measure the length and breadth of two cells. Record these measurements in your diagram.

Notes on procedure

- Students should be shown how to cut the stems in a motion away from fingers to reduce risks of cuts. Woody or tough stems should not be used. If stems are small it may be helpful to use a piece of pith (such as a carrot) with a notch cut out, placed on top of the stem to hold it steady. Alternatively, the material for sectioning could be held within a celery stem.
- Students often lack confidence in the correct use of microscopes. The microscopes used at A level may be different to those used lower down the school. It is worth spending a few minutes to review how to use and care for the microscopes at the start of the lesson.
- Make sure that razor blades are counted in.

Answers to questions

1. This will depend on the size of the drawing and the objective used. Students should divide the image size (length of scale bar measured with a ruler, converted to μm) by the actual size (the length that the scale bar represents in μm).
2. A suitable summary might include the following points.
   - Use a stage micrometer and eyepiece graticule (eyepiece micrometer).
   - Calibrate the eyepiece graticule with the objective lens that will be used.
   - Do this by measuring the eyepiece scale against the scale of the stage micrometer.
   - Measure the cell using the eyepiece scale and convert into length units (μm).
3. Calculate a mean. Measure more cells to estimate a more reliable mean. The volume of the cells would be a better descriptor of size than the linear dimensions.

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All the maths you need

- Recognise and make use of appropriate units in calculations.
- Recognise and use expressions in decimal and standard form.
- Use an appropriate number of significant figures.
- Make order of magnitude calculations.
- Change the subject of an equation.
- Substitute numerical values into algebraic equations using appropriate units for physical quantities.
- Solve algebraic equations.
- Calculate the circumferences, surface areas and volumes of regular shapes.

Equipment

- plant stem, at least 5 cm long
- microscope with an eyepiece graticule
- stage micrometer slide
- toluidine blue O stain
- two glass microscope slides
- two coverslips
- small paintbrush or tweezers
- single-sided razor blade
- white tile
- watch glass
- dropping pipette
- mounted needle
- lens tissue
- absorbent paper (e.g. paper towel)

Diagrams

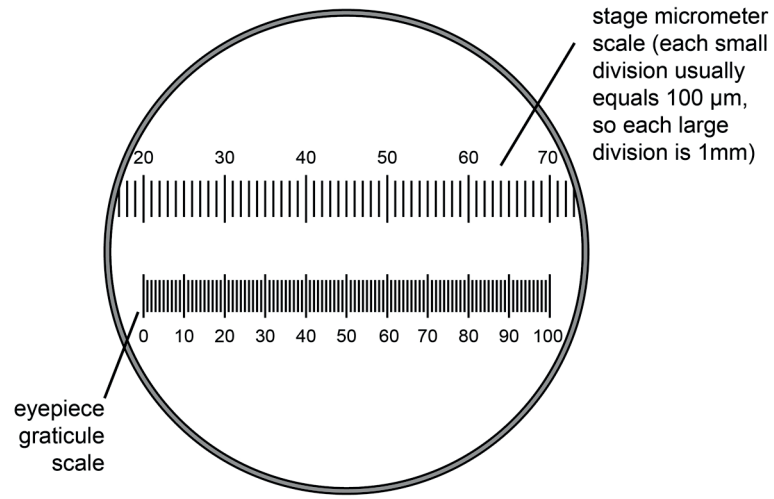
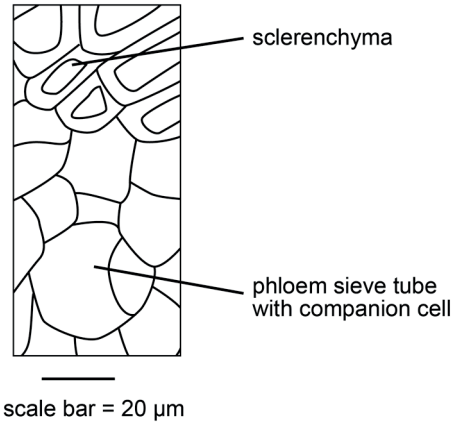


fig A Calibrating the eyepiece graticule using a stage micrometer.



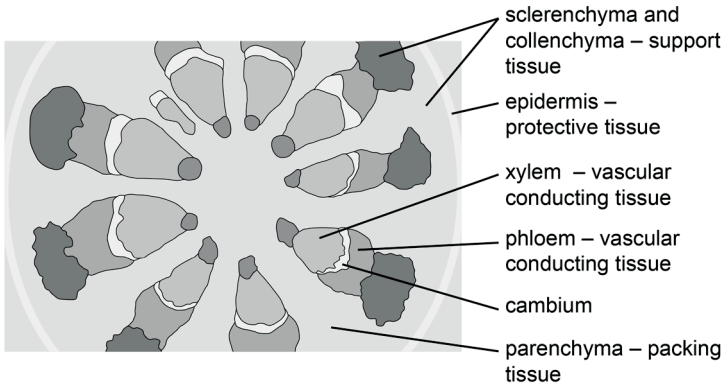


fig B Transverse section of a stem as seen through a microscope, with drawings at high and low power.

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Learning tips

- To be able to answer examination questions about the magnification of images, make sure that you learn the equation: $\text{magnification} = \frac{\text{size of image}}{\text{size of real object}}$.
- You should know how to rearrange the magnification formula to calculate any of the values. Remember to convert all lengths to the same units, usually μm.

Tips for good biological drawing:

- Use a sharp HB pencil. Keep lines clear and continuous, not feathery or sketched.
- Draw only what you see. Do not draw stylised patterns, and do not make it up!
- Start with an outline. Keep it large and think about proportions.
- Do not use shading or colour.
- Draw label lines in pencil with a ruler. Lines should not have arrowheads and should just touch the item to be labelled.

Questions

1. Using the scale bar that you have drawn on your detailed drawing of cells, work out the magnification of your drawing.
2. Write a four-point summary to explain how cells can be measured using a microscope.
3. You were asked to measure the length and breadth of two cells. Suggest improvements to this method of describing the size of cells in a tissue.

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- Take care when using and carrying razor blades. Provide a sharps box for the safe disposal of razor blades, details on sharps disposal can be found in CLEAPSS Bulletin 154 (Autumn 2015).
- Use disposable gloves and eye protection.
- Avoid inhaling particles of the toluidine blue O solution. You may wish to prepare it in a fume cupboard.

Equipment per student/group	Notes on equipment
plant stem	The specimen should be at least 5 cm long. There are many suitable species. Those from the genera *Pelargonium* (geranium), *Impatiens* (busy lizzie), *Ranunculus* (buttercup) and *Helianthus* (sunflower) work well, while bean, privet and celery can make useful comparisons.
microscope with an eyepiece graticule	School microscopes with ×400 maximum magnification are usually suitable. Graticules should be placed in the eyepiece lens by a technician and not by students to avoid damage to the microscope.
stage micrometer slide	Eyepiece graticules and stage micrometers are expensive; simple versions can be made from film strip with scales, available from some suppliers.
toluidine blue O stain	0.05% aqueous solution. It may be necessary to use a slightly higher concentration of toluidine blue solution (up to 0.5%) with some plant tissues, but over-staining gives poor differentiation between the colours. Making the stain up in pH 7 buffer solution may also enhance staining. Provide gloves for students to use while carrying out the staining.
glass microscope slides	Two per group
coverslips	Two per group
small paintbrush or tweezers	One per group

single-sided razor blade	These must be sharp and very clean, new if possible, as any trace of corrosion or organic matter introduces an infection concern if skin is broken by blade. Provide a sharps box to receive used razor blades.
white tile	One per group
watch glass	One per group
dropping pipette	One per group
mounted needle	One per group
lens tissue	One per group
absorbent paper (e.g. paper towel)	One per group

Notes