

## Appendix 2 – practicals for which full details are provided

### Practical 1 - Investigation into size and scale of microscopic tissues

This practical focuses on microscope technique and using graticules and stage micrometers to determine size and scale in biological cells and tissues.

#### Intended learning outcomes

By the end of this practical you should be able to:

- Use a microscope fitted with an eyepiece graticule and stage micrometer
- Calibrate the eyepiece graticule using the stage micrometer
- Use the calibrated graticule to determine the actual size of microscopic specimens
- estimate the accuracy of a measurement
- Use the graticule to determine scales
- Understand the importance of repeating or validating set of results.

#### Safety Information

There are no particular hazards in this practical, however you must follow your laboratory rules.

#### Background information

- The measurement of specimen size with a microscope, is made by using an eyepiece graticule. This is a glass or plastic disc with 8 divisions etched onto its surface, which is inserted into the eyepiece lens.
- The size of the eyepiece graticule remains constant, despite the fact that the image viewed will change its size depending upon whether high- or low-power objective lenses are used. For example a cell viewed with the x40 objective will appear much larger than when viewed with the x10 objective. However because the graticule is in the eyepiece it will not change its size. Therefore the value of each of the divisions in the eyepiece graticule varies with the magnification of the objective lens.
- A stage micrometer is a very accurately etched glass or plastic ruler that is placed on the microscope stage so that the eyepiece graticule scale is superimposed on the stage micrometer scale. The scale is usually 1mm divided into 100 separate divisions so that each division equals 10 micrometres (10 $\mu$ m).
- It is necessary to calibrate the eyepiece graticule with the stage micrometer placed on the microscope stage for each objective lens used.

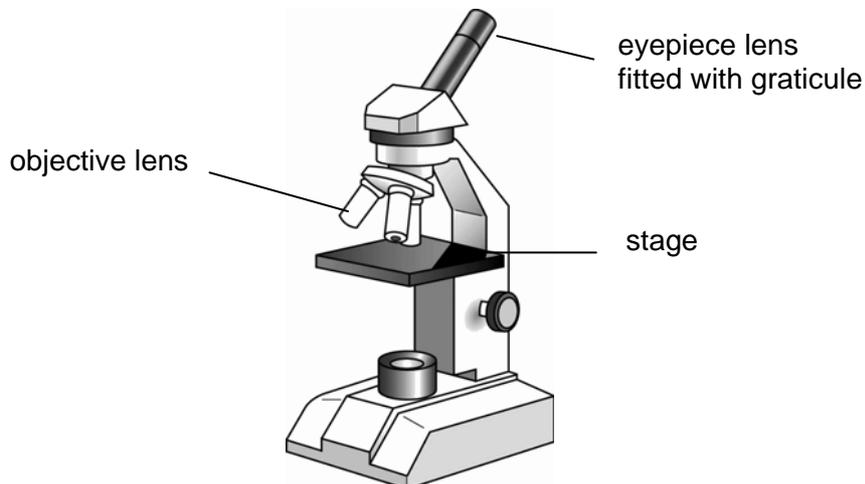
You will observe a TS of plant tissues through a microscope and use an eyepiece graticule and a stage micrometer to determine the size of some of the structures.

- Read the information above.
- Ensure that you understand the principles of using an eyepiece graticule and a stage micrometer before you continue with the investigation.

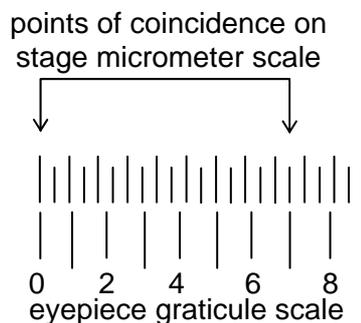
## Method

### Preparation

1. You have been provided with a compound light microscope with both low- and high-power objective lenses and an eyepiece lens that has been fitted with a graticule. You have also been provided with a stage micrometer.



2. You must now calibrate the eyepiece graticule. Place the stage micrometer onto the microscope stage and focus using the low-power objective lens so that the graticule scale becomes superimposed over the stage micrometer scale.



3. Move the stage micrometer until the start or zero line of each scale is coincident (lined up)
4. Look along the scale until another coincident point is found.
5. The relationship between the two scales can now be calculated

On the scale shown there are 17 divisions on the stage micrometer scale that line up with 7 divisions on the graticule scale.

Thus  $17 / 7 = 2.42857$  units.

Each unit on the stage micrometer scale is 10 micrometres ( $10\mu\text{m}$ ).

Therefore each division on the graticule scale is 24.2857 micrometres rounded to  $24.3\mu\text{m}$ .

6. Use the procedure described above to determine the size of each division on the eyepiece graticule using the low-power objective lens of your microscope.
7. Repeat the procedure to determine the size of each division when using the high-power objective lens.

### Making observations

1. You are provided with a stained transverse section through part of a dicotyledonous plant root.
2. Examine the specimen using the low-power of your microscope.
3. Make a large, **plan** drawing to show the distribution of tissues, labelling the stele (vascular bundle).
4. Use the eyepiece graticule to measure the width of the vascular bundle at its widest point in graticule units and then calculate the actual width of the vascular bundle in millimetres and in micrometres.
5. Draw a straight line on your drawing across the vascular bundle to show where you took your measurement. Write the dimension on your drawing next to the line.
6. Make a high-power drawing to show a group of **four** xylem vessels from inside the vascular bundle.
7. Use the eyepiece graticule to measure the width of the xylem vessel at its widest point in graticule units and then calculate the actual width of the vessel in micrometres, remembering to use the appropriate calibration of the eyepiece graticule for the high-power objective lens.
8. Draw a straight line on your drawing across the xylem vessel to show where you took your measurement. Write the dimension on your drawing next to the line.
9. Look at your two measurements and check on their accuracy. The actual size of the xylem vessel should be smaller than the size of the vascular bundle even though it looked larger using the high power objective lens.
10. You are now going to determine the magnification of your drawing of the xylem vessels. Use a ruler to measure the length of the line that you drew across the xylem vessel.

Use your knowledge of the actual size of the vessel to calculate the magnification of your drawing. Write your answer  $\times$ \_\_\_\_\_ at the bottom right hand corner of your drawing.

### Follow-up

- Compare your results with other members of the class and check for consistency of readings.
- Did any member of the class have anomalous results? What are the potential causes of such an anomalous result in this investigation?
- Write up your procedure including a discussion of the benefits of comparing your results with other students.

## Practical 1 - Lesson Plan

### Investigation into size and scale of microscopic tissues.

#### Context

A practical investigation set in the context of 9700 syllabus – Assessment objective C 2 and 3 – Cell structure.

#### Key aims of the lesson

This practical is designed to develop the skills of using an eyepiece graticule and a stage micrometer with a microscope to determine size and scale of microscopic tissues.

#### Intended learning outcomes

By the end of the practical and the write-up the student should be able to

- Use a compound light microscope with an eyepiece graticule
- Use a compound microscope with a stage micrometer
- Calibrate an eyepiece graticule with a stage micrometer
- Use the calibrated graticule to determine the actual sizes of cells and tissues using both low- and high-power objective lenses.

#### Resources required

White board or flipchart and suitable pens or blackboard and chalk

Practical materials specified on the Technical Information Sheet.

Copies of the student worksheets.

#### Planned activities

Timings/ minutes	Teacher/ Student Activities
End of previous lesson	<b>Preparation</b> – Student worksheet given out for students to read in preparation for the practical lesson.
0 - 3	<b>Introduction</b> to the aims, intended outcomes and shape of the lesson – teacher led oral presentation
3 - 10	<b>Context</b> – review the method for setting up and using a microscope with an eyepiece graticule and stage micrometer and how to calibrate the eyepiece graticule.
10 - 15	<b>Introduction to method</b> – Teacher briefly outlines method and answers any student questions on procedure. Teacher emphasises safety concerns regarding breaking slide by focussing down with the objective lens.

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15 - 40	<b>Carrying out the practical</b> – students carry out the practical work.
40 - 50	<b>Obtain results</b> – Students compare their results with other students in the class
50 - 60	<b>Drawing together the threads</b> – Teacher led discussion on the skills that have been developed as well as discussion on results obtained.

### **Practical 1 - Technical information**

#### **Investigation into size and scale of microscopic tissues.**

The apparatus and materials required for this practical are listed below.

The amount of apparatus listed is for one student or one group of students if they are to work in groups.

1. 1 x prepared slide TS *Ranunculus* root
2. 1 x microscope with an eyepiece graticule fitted, suitable illumination and:
  - high power objective lens e.g. x40 (equal to 4mm or 1/6")
  - low power objective lens e.g. x10 (equal to 16mm or 2/3")
3. 1 x stage micrometer – a scale on a glass slide
4. suitable white paper, HB (medium-hard) pencil and rubber

#### Safety Precautions.

No specific hazards have been identified in this practical, however a risk assessment should be carried out as a matter of course.