Teaching microscopy using plants: calibrating and measuring

Chris Graham suggests activities for developing these important skills in plant biology

Being able to measure objects seen under a microscope can help students gain a better sense of scale at the cellular level and become more aware of the nature of the subcellular world. It also provides opportunities for students to collect data to explore fascinating questions.

Whereas my previous 'Teaching microscopy using plants' articles (Graham, 2024, 2025) discuss the development of microscopy skills suitable for students across the whole 11 to 18 age range, this article focuses on skills that tend to be required of older students (e.g. 16- to 18-year-old students studying A-level biology in England or students in FE colleges). However, with minor modifications, most of the preparations discussed in this article could be used with younger students as well.

This article suggests four activities to support the development of calibrating and measuring skills (see Box 1 for the definition of key terms). To allow as much time as possible for developing these skills, the specimens suggested here require minimal preparation.

The first suggested activity uses printed images of a pre-prepared transverse leaf section (such as the sunflower *Helianthus annuus*), a stage micrometer and an eyepiece graticule as a paper-based exercise (Figure 1a). This activity teaches students the skills of calibrating and measuring without imposing the complexities of microscopy on the task. The other three activities provide teachers with options for tasks that require students to practise their calibrating and measuring skills while answering interesting biological questions. Each activity explores a different aspect of measurement (length, area or speed) and encourages students to consider aspects of biology beyond just microscopy. The second activity, the often-used onion (*Allium cepa*) epidermis (Figure 1b,c), involves measuring the width of cells in different onion layers to explore how onions grow. Growth continues to be the exploratory theme in the third activity, which asks students to measure stomatal density in leaves of the silver inch plant (*Tradescantia zebrina*) (Figure 1d,e). By comparing larger, older leaves with smaller, younger ones, students can explore what changes occur as leaves grow. Finally, students get a sense of 'what counts as fast' at a subcellular level by measuring the speed of chloroplast movement in the pondweed *Egeria densa* (Figure 1f,g). Full methods for these activities can be found on the Science and Plants for Schools (SAPS) website (see *Useful links*).

Box 1 Calibrating and measuring: key terms

Eyepiece graticule – a scale that sits in the eyepiece.

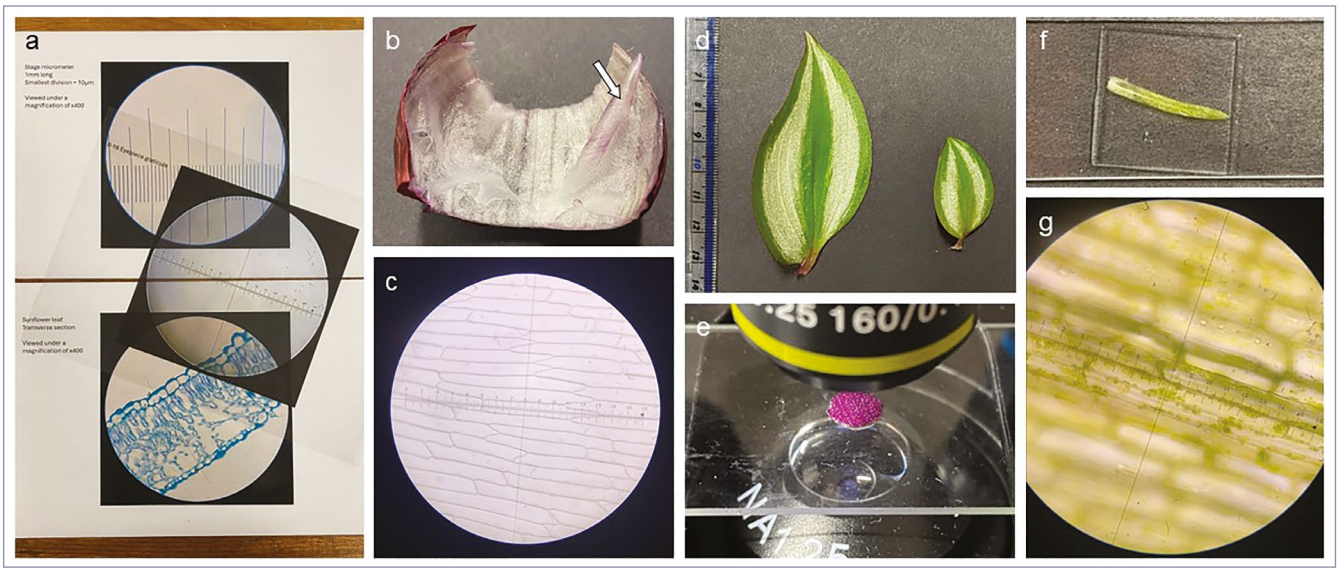
Stage micrometer – a 'mini-ruler' on a slide.

Calibrating – using a stage micrometer to measure the length of one division on the eyepiece graticule at a particular magnification.

Measuring – measuring the size of objects under the microscope in 'eyepiece units' (EPUs) and then multiplying this by the number of micrometres each EPU represents at that magnification.

Calibrating and measuring using a sunflower (*Helianthus annuus*) leaf section

All students are familiar with leaves, and post-16 students should have a clear understanding of their



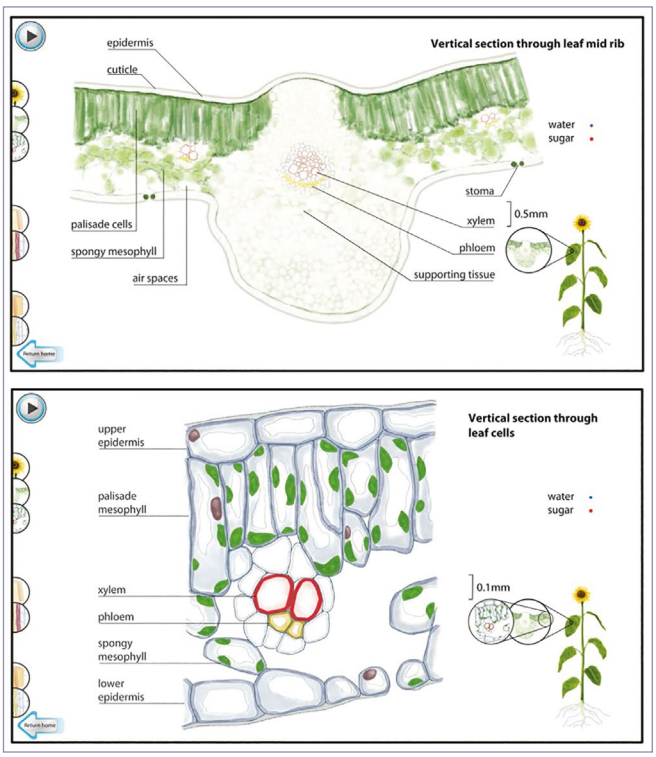
▲ **Figure 1a** Paper-based resources for calibrating and measuring leaf thickness; **1b** the outermost layer of an onion with its inner (adaxial) epidermal layer [arrow]; **1c** the epidermal layer of onion cells (mag ×100, field of view ≈2000 µm); **1d** two leaves of *Tradescantia zebrina*, an older, larger one and a smaller, younger one; **1e** a hole punched disc from the larger *Tradescantia* leaf; **1f** a leaf from the pondweed *Egeria densa* on a slide; **1g** a still from a video of chloroplast movement in this *Egeria densa* leaf (mag ×400, field of view ≈450 µm)

structure and function. Asking students to estimate the thickness of a leaf in millimetres and then convert it into micrometres provides a useful opportunity to check whether students need support with converting between units.

Some students find the concept of calibrating a microscope a real challenge and so removing the use of the microscope itself when teaching this skill for the first time can be very beneficial (e.g. Masters, 2024). Students can be provided with copies of the view down a microscope of a transverse leaf section (e.g. the sunflower *Helianthus annuus*) at ×40, ×100 and ×400 (see *Useful links* to download free copies), and animations on the Science and Plants for Schools website (see *Useful links*) can help ensure students are familiar with what they are looking at (Figure 2).

Students measure leaf thickness in 'eyepiece units' (EPUs) at an identified location on each image, using the image of an eyepiece graticule printed on clear acetate film (Figure 3a). The thickness of the leaf in EPUs varies depending on the magnification of the image. This allows students to see that EPUs are not fixed-distance measurements and that the distance they represent for each magnification needs to be calculated. This is the process of calibration.

Students can then use the images of a stage micrometer, at ×40, ×100 and ×400, to calculate how many micrometres each EPU represents at each



▲ **Figure 2** Screenshots from the 'Transport of water and sugar in plants' section of the 'Plant Biology' animation on the Science and Plants for Schools website (see *Useful links*)

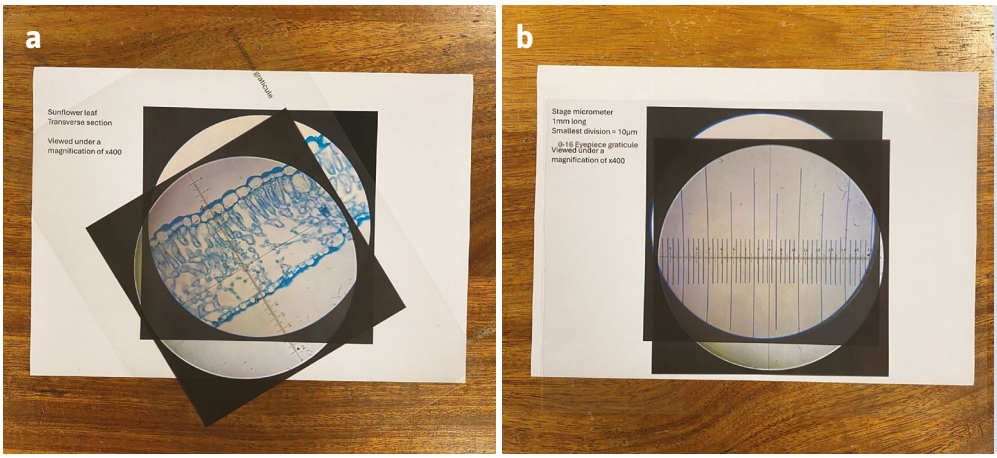


Figure 3 A paper-based activity for developing calibrating and measuring skills: **a** measuring the thickness of a leaf; **b** calibrating the eyepiece graticule

magnification (Figure 3b). Multiplying the number of micrometres each EPU represents by the thickness of the leaf in EPUs allows the students to calculate the thickness of the leaf. All students do this with the same images and measure thickness at the same point on the image, so they should all come up with very similar answers. This makes it easy to identify students' errors.

Some students may move through this task very quickly and others may need much more support. To allow time for the teacher to support those who need it, the other students could do the same process for 'real' using a microscope, collating repeated measurements of leaf thickness to obtain a more accurate value for the thickness of a leaf. To further develop expertise, students could be asked to move on to measure the size of different cell types, or the diameter of a nucleus or a chloroplast.

Exploring cell size in red onion (*Allium cepa*) inner (adaxial) epidermis

Using the skills developed through the 'thickness of a leaf' activity, students can investigate how plants grow by exploring the size of the cells of the inner (adaxial) epidermis of red onion (*Allium cepa*) layers (Figure 1b). The base of an onion is a flattened stem and the layers are fleshy modified leaves adapted for storage. Because the stem is so compact, the modified leaves all lie next to each other, forming the onion bulb, instead of being spread out as in a normal shoot (for further reading about onions and bulbs in general see *Useful links*). The outer layers of the bulb are bigger leaves that have grown as the onion has grown, and the inner layers are younger, smaller leaves that are yet to grow as big. This means that an onion provides examples of leaves at different stages of growth for students to explore. By measuring cell length and width as well as the size of the leaves themselves, students can answer a variety of questions about plant growth such as:

1. Do bigger leaves have bigger cells?
2. Do the cells have the same length:width ratio across the different leaf sizes?
3. Is the growth of leaves due to an increase in cell size, the production of new cells or both?

The inner epidermis of an onion layer is easily peeled off and mounted in water under a coverslip. No staining is required for this activity.

The layers of an onion can be numbered, with the innermost layer used being 'layer 1' going up to the outermost layer (Figure 4). Each student or pair of students could be given a sample from one of the layers of the onion to measure the length and width of a selection of cells (at magnifications of ×40 or ×100) in EPUs (Figure 5). Once the measurements are done, students can then calibrate the eyepiece graticule for the magnification used and convert their cell measurements into micrometres.

Collating data from a whole class allows graphs to be plotted such as the ones in Figure 6. These data allow questions 1 and 2 in the list above to be answered.

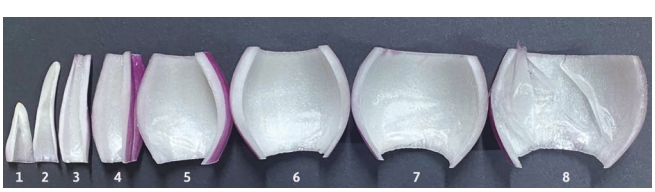
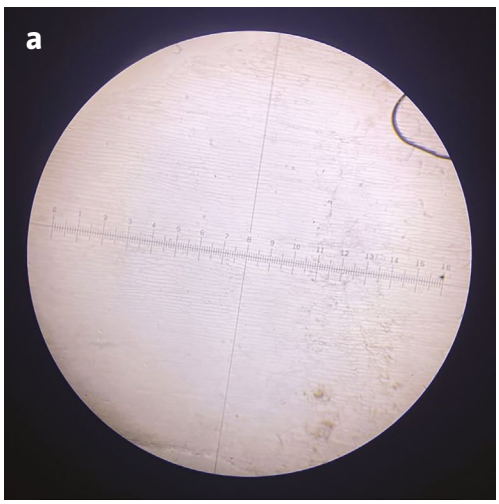


Figure 4 Separated layers of a red onion (*Allium cepa*) numbered from smallest to largest



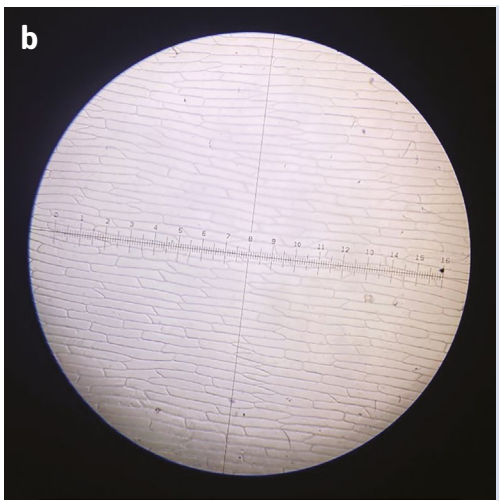


Figure 5a Cells from layer 1 of the onion (mag ×40, field of view ≈4500 µm); **5b** cells from layer 8 of the onion (mag ×40, field of view ≈4500 µm)

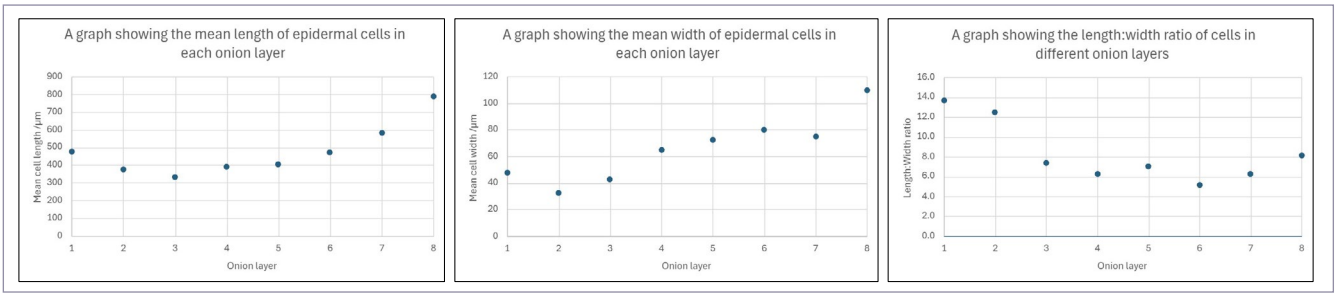


Figure 6 Graphs of onion epidermal cell length, width, and length:width ratio

To determine whether the growth of leaves is due to an increase in cell size, the production of new cells or both (question 3), an estimate of the size of each leaf is needed alongside the size of the cells in each leaf. The cells in the epidermis are orientated such that the longer cell dimension (the 'length') aligns with the tip-to-base axis of the leaf, and the shorter dimension (the 'width') aligns with the circumference of the onion layer. The easiest way to answer question 3 is to focus on the width dimension. Assuming each onion layer is circular in cross-section, the diameter of each layer's widest point can be measured after cutting the onion in half (Figure 7), and the circumference calculated before samples are distributed to students. This, alongside the mean width of cells in each layer, can be used to calculate how many cells each layer has around its widest point. This identifies whether mitosis has occurred, and the previous measurements show whether cells have increased in size, thereby allowing question 3 to be answered.

If using this activity with younger students, take care to avoid creating or reinforcing the misconception that growth is due to an increase in cell size rather than an increase in cell number. In pre-16 lessons, students are expected to recall that growth is due to an increase in cell number; however, in post-16 lessons there are topics where an understanding of the role of an increase in cell size is important. Probably the most significant context in this regard is the increase in cell size on the shaded side of a shoot associated with phototropism.

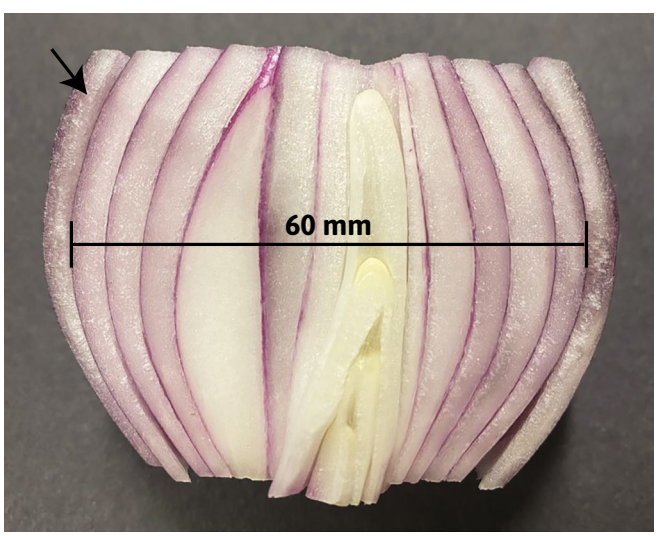


Figure 7 Measuring the diameter of the inner epidermis of one onion layer (arrow)

Exploring stomatal density in the silver inch plant (*Tradescantia zebrina*)

The onion epidermis activity explores growth in a tissue of relatively uniform cells. However, tissues often have a variety of differentiated cell types. In the lower epidermis of a *Tradescantia zebrina* leaf there are green guard cells around stomata, and green pigmented epidermal cells around the guard cells. The remaining epidermal cells contain a purple pigment. This specimen provides students with an easy way to measure stomatal density and answer questions such as:

1. Do leaves of different sizes on the same plant have the same stomatal density?
2. Do leaves make more stomata as they grow?

To provide samples from large and small leaves for a whole class, a hole punch could be used to cut several leaf discs from each leaf. No coverslip is required and each leaf disc just needs gently pressing onto a slide to ensure it is held in place before viewing (Figure 1e). A magnification of ×100 (using a ×10 eyepiece and a ×10 objective lens) is appropriate for this activity and students would measure the radius of their field of view at ×100 using a stage micrometer first. This allows the area of the field of view to be calculated, after which counting the number of stomata in the field of view allows the density of stomata per mm^2 to be calculated (Figure 8).

Measuring the length of each leaf before leaf discs are cut shows how many times bigger the larger, older leaves are than the smaller, younger ones; assuming the proportions remain the same, this can be used to estimate the number of times greater the surface area of a large leaf is compared with a small leaf. This increase in surface area can be compared with any change in stomatal density to determine whether more stomata have been produced as the leaves grow.

Exploring the speed of chloroplast movement in the pondweed *Egeria densa*

Cytoplasmic streaming occurs in many plant cells, particularly large ones. It is thought to increase the rate of transport of molecules and vesicles within these cells (Verchot-Lubicz and Goldstein, 2010) and the most obvious visible effect of it in some cells is the movement of chloroplasts. Another suggested benefit of cytoplasmic streaming is that it increases the rate of photosynthesis of the cell under patchy light conditions owing to the redistribution of molecules between areas dominated by respiration and those dominated by photosynthesis (Dodonova and Bulychev, 2012).

Mounting a single leaf of the pondweed *Egeria densa* in water under a coverslip (see Graham, 2024) and viewing at ×400 magnification allows moving chloroplasts to be seen. If the pondweed is kept well lit and at room temperature in advance, there is a very good chance of seeing moving chloroplasts in most cells of the leaf.

Using an eyepiece graticule and a stopclock allows the speed of chloroplast movement in eyepiece units (EPUs) to be determined (Figure 9). Calibrating the eyepiece graticule with a stage micrometer allows this to be converted into micrometres per second. Before calibrating, it is worth asking students to have a guess at how long it would take a chloroplast to travel 1 m. It is much longer than students normally imagine. The chloroplasts look as though they are moving quickly but they are only travelling very small distances. This shows that organelles (or even diffusing molecules) do not have to move very quickly to travel around a whole cell in a relatively short period of time. It also helps give students a sense that our feeling for the world at our scale does not translate well to the microscopic world.

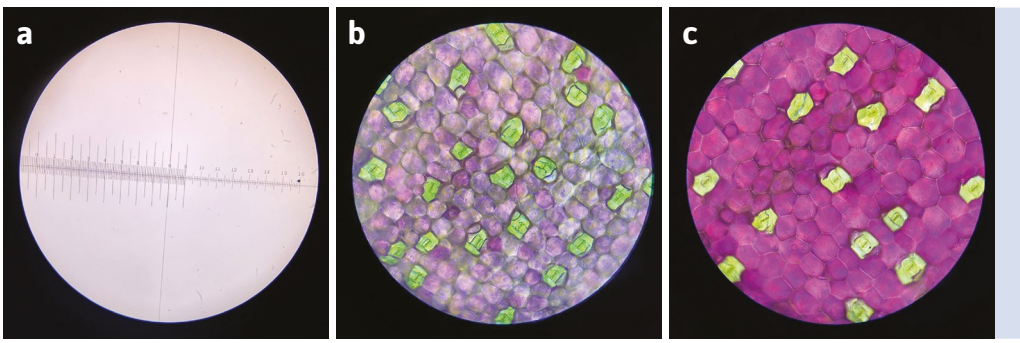


Figure 8 Exploring stomatal density: **a** calculating the area of the field of view at ×100 magnification [$r = 0.9$ mm, $\pi r^2 = 2.54$ mm^2]; **b** a small leaf [8.3 stomata/mm^2]; **c** a large leaf [5.1 stomata/mm^2]

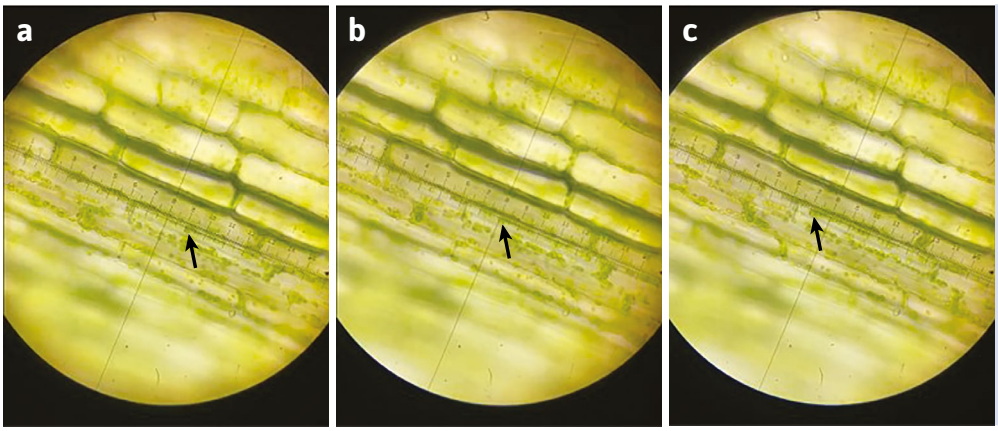


Figure 9 Stills from a video of moving chloroplasts; the arrow identifies the same chloroplast in each image, which was moving at 6.4 µm/s: **a** $t = 0.0$ s; **b** $t = 3.9$ s; **c** $t = 8.0$ s

Summary

By using a paper-based activity to develop the skills of calibrating and measuring in a familiar specimen, students have a greater capacity to focus on gaining these potentially challenging skills. The microscope-based activities then provide opportunities for students to practise their calibrating and measuring skills. Asking questions about growth provides an interesting context for this practice, and exploring the movement of chloroplasts challenges students' perceptions about the subcellular world.

The variety of activities suggested also allows a range of different maths skills to be practised and developed, including calculating areas, ratios, density (i.e. stomata per mm^2) and speed. This could even be extended to conducting statistical tests, such as using the Spearman's rank correlation coefficient to see whether cell size correlates with layer number in onions.

If you would like free access to full methods for these activities, high resolution copies of the images in this article and some associated videos for use in your teaching, please visit the Science and Plants for Schools (SAPS) website (see *Useful links*).

All photographs were taken by the author using a handheld iPhone 11. The microscope used was a VWR Visiscope series 200 Model TL224 with a ×10 eyepiece and ×4, ×10 and ×40 objective lenses.

USEFUL LINKS

Science and Plants for Schools (SAPS) *Teaching microscopy using plants*: www.saps.org.uk/microscopy-ssr

Science and Plants for Schools (SAPS) *Transport of water and sugar in plants – animation*: www.saps.org.uk/transport-animation

The Herb Society, *Herb Histories: Onions – The ABCs of* Allium cepa: https://herbsociety.org.uk/2020/09/19/herb-histories-onions-the-abcs-of-allium-cepa

Britannica, *Bulb*: https://www.britannica.com/science/bulb

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