**The dissection of a stem STUDENT**

**Introduction**

In this activity, you will make thin sections of plant tissue, stain them and view them under the microscope. You will be able to identify different differentiated cells as well as practising your dissection and microscopy skills.

In a long, thin specimen, such as a stem, transverse sections are made by cutting perpendicularly across. Longitudinal sections are made by cutting along the line of the specimen.

**Aims**

To use dissection tools safely and effectively to produce stained sections of celery (*Apium graveolens* var *dulce*) and to observe and draw these using a microscope.

**Intended class time**

* 1 hour

**Equipment**

* Microscope
* Microscope slides
* Coverslips
* Fresh head of celery
* 0.5% Toluidine blue
* Scalpel or single edged razor blade
* White tile
* Forceps
* Dropping pipettes
* Tap water
* Eyepiece graticule
* Stage micrometer
* Watch glass

**Method**

*Note: take care when using the sharp dissecting instruments.*

*Note: The table below shows the colours that you should expect to see in your preparation. Generally non-lignified tissue should be pink/purple and lignified tissue should be green/blue. Both colours tend towards dark blue when over stained.*

|  |  |
| --- | --- |
| **Tissue Element or Structure** | **Colour** |
| Xylem | Green or Blue-green |
| Phloem | Red |
| Sclerenchyma | Blue-green, sometimes Green |
| Collenchyma | Red-Purple |
| Parenchyma | Red-Purple |

*(From Parker, A. J., Haskins, E. F. and Deyrup-Olsen, I. (1982) Toluidine Blue: A simple, Effective Stain for Plant Tissues. The American Biology Teacher., Vol. 44, No. 8, pp. 487-489)*

Transverse Sections

1. Obtain a stick of celery (*Apium graveolens* var *dulce*) about 5 cm long.
2. Rest the stem horizontally on a white tile and use a blade to cut one end as perpendicular to the length of the stem as possible.
3. Now use the blade to cut very thin perpendicular slices (transverse sections) of the celery from the edge you have just cut. Do not discard the remaining celery stem – you will take further sections later.
4. Use forceps to gently lift the transverse sections into a small beaker containing tap water and leave to soak for 2 minutes.
5. Use a stage micrometer to calibrate the eyepiece graticule for use with x4, x10 and x40 objective lenses. Make a note of each calibration for later use.
6. Use forceps to gently lift the transverse sections into a watch glass containing toluidine blue and leave them in the stain for 1 minute.
7. Use forceps to gently lift the transverse sections back into tap water to rinse off excess stain.
8. Place a transverse section on a microscope slide. Add a drop of tap water and a coverslip. Repeat this for your three thinnest transverse sections.
9. View under the lowest magnification (x4 objective lens).
10. Find the clearest view that shows a variety of structures within the stem and produce a scientific drawing of what you see. Use the graticule and your previously noted x4 objective calibration factor to add a scale bar and work out the magnification of your drawing.
11. View under a higher magnification objective lens (x10 objective lens) and find the clearest view that shows one vascular bundle.
12. Produce a scientific drawing of what you see. Use the graticule and the relevant calibration factor to add a scale bar and work out the magnification of your drawing.

Longitudinal Sections

1. Take the remaining celery stem. Just as in step 2 above cut a short piece off the end perpendicularly to remove the part that has dried out while you have been working on the transverse sections.
2. Make another perpendicular cut to produce a piece of stem about 2 cm long.
3. Carefully cut the piece of stem in half lengthways i.e. split it down the middle.
4. Now use the blade to cut very thin lengthways slices (longitudinal sections) of one of the split halves, starting from the freshly cut inner surface.
5. Use forceps to gently lift the longitudinal sections into a small beaker containing tap water and leave to soak for 2 minutes.
6. Repeat steps 6 to 12 from the transverse sections method with the longitudinal sections.

**Extension questions**

1. Why is it important to produce very thin slices of plant tissue?
2. Why is it important that the sections are truly transverse (or longitudinal) and not cut at an angle?
3. What did you find was the best way to get sections as thin as possible?
4. Why are stains useful in microscopy?
5. Why is Toluidine blue useful for this protocol?

**To submit**

For this piece of work to count towards Practical Activity Group 2 of the Practical Endorsement, you should have your annotated drawings of the transverse and longitudinal sections. You also need to have considered the above questions as the answers to these questions will aid you in preparation for your written examinations.