Leaf Epidermal Tears

Introduction:
Like the Onion Epidermal cells exercise this one gives a tissue that is one cell thick and thus easy to focus under the microscope.

The epidermal layer of leaves consists of flat cells that have a protective function. They are transparent (because they lack chloroplasts) so that light will be transmitted to the underlying photosynthetic tissues (palisade mesophyll and spongy mesophyll).

When doing the actual 'tear' you will look for this feature. If you have a layer of colourless near-transparent cells then you have separated the epidermis. If your tear has green areas then the epidermis has not separated and you will need to repeat the 'tearing' process.

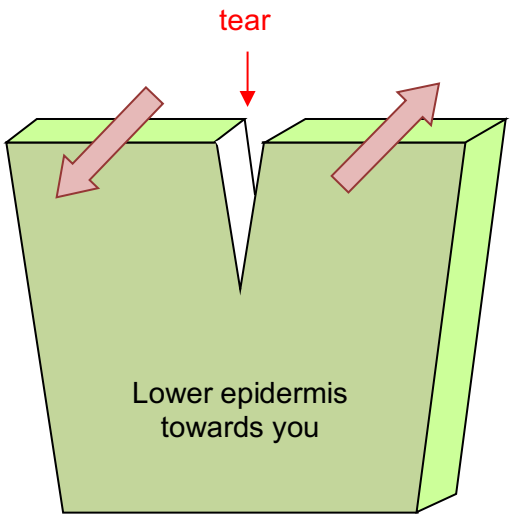
There are two epidermal layers (upper and lower) but the lower one is generally of more interest because it usually contains stomata. The epidermis secretes a waxy cuticle, which serves to make the leaf waterproof and protect it from desiccation. As well as being impervious to water however the wax is also impervious to gases like oxygen and carbon dioxide. Because these gases need to move in and out of the leaf during photosynthesis, the lower epidermis has small pores called stomata (singular, stoma). Opening and closing these stomata is the function of the guard cells.

Method

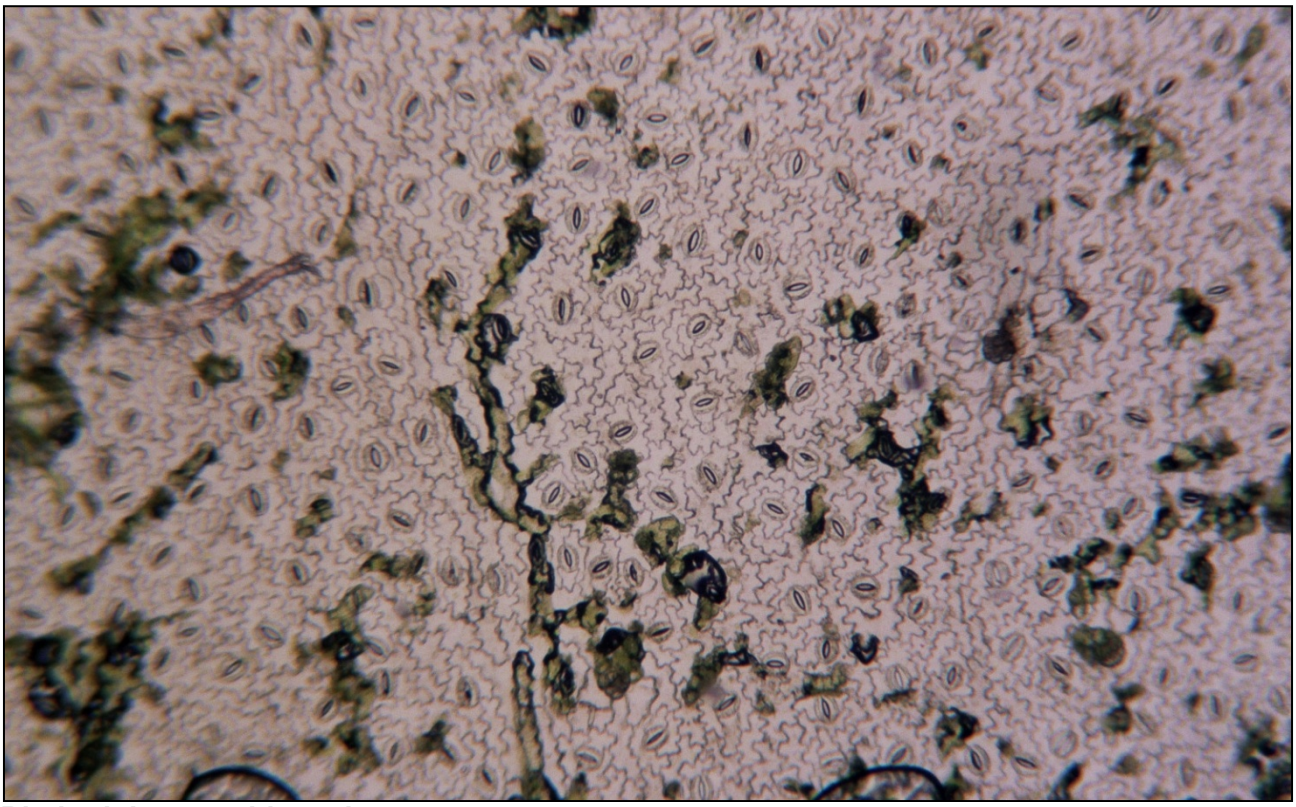
Choice of leaf material.
For this exercise soft herbaceous leaves are preferred. They must rip easily but more importantly the lower epidermis must separate from the spongy mesophyll cells. Traditionally rhubarb has been used but there are many plants that will give satisfactory results. Examples include broad beans (grow your own in the lab), dandelions, radish, agapanthus and even the young leaves of roses.

The tear

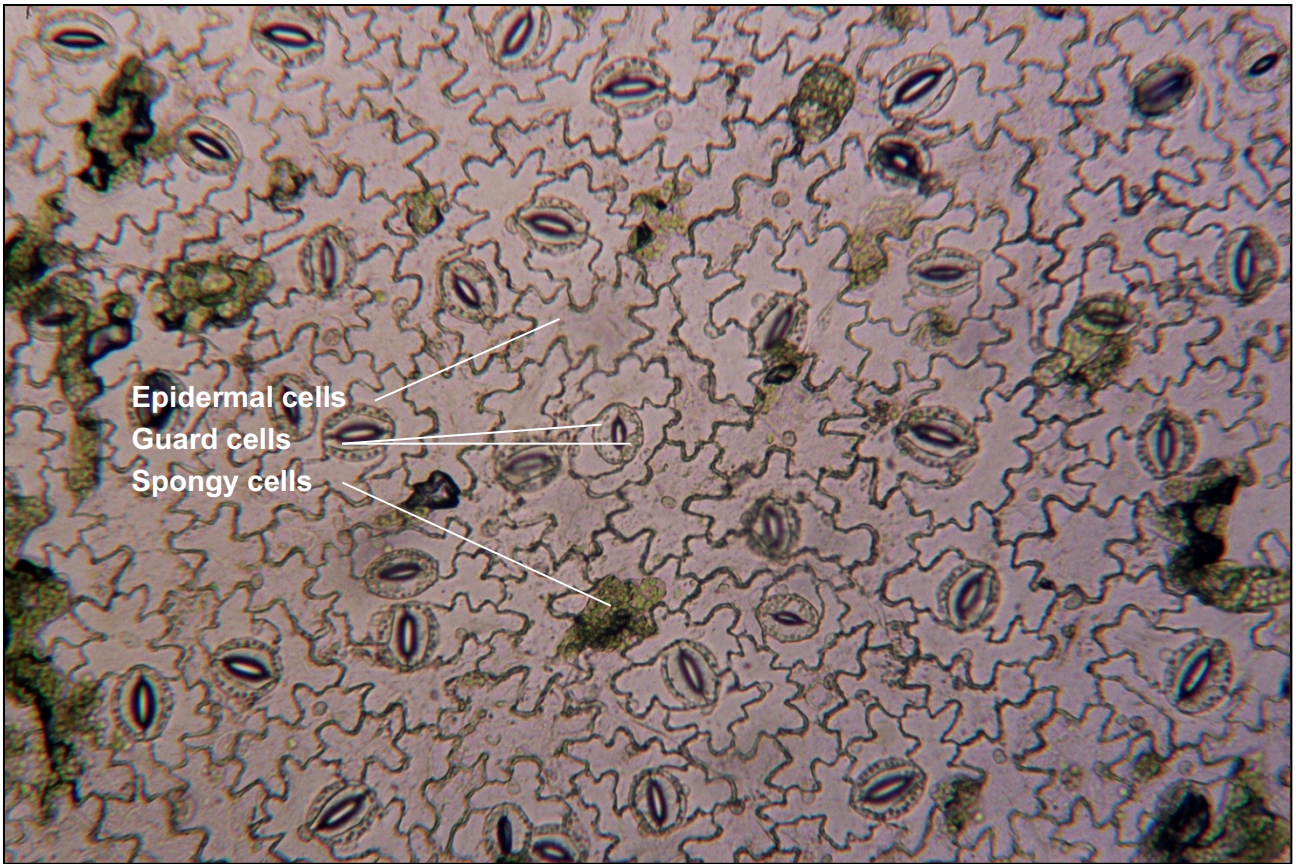
- Have a slide ready with a drop of water. You can use stain (iodine if you wish but use water initially)
- Cut a section of leaf that does not contain any large veins
- Hold the leaf with the lower epidermis towards you, between the forefinger and thumb of both hands
- Move your hands in opposite directions so that the leaf begins to tear as shown below:
- As the 2 parts of the leaf separate look for a small transparent layer of cells
- Cut this section of epidermis away with fine scissors and mount it in the water on your slide
- Add another drop of water (or stain) on top of the epidermal layer and lower a cover slip. Use a small piece of absorbent paper to mop up any excess fluid



Images:



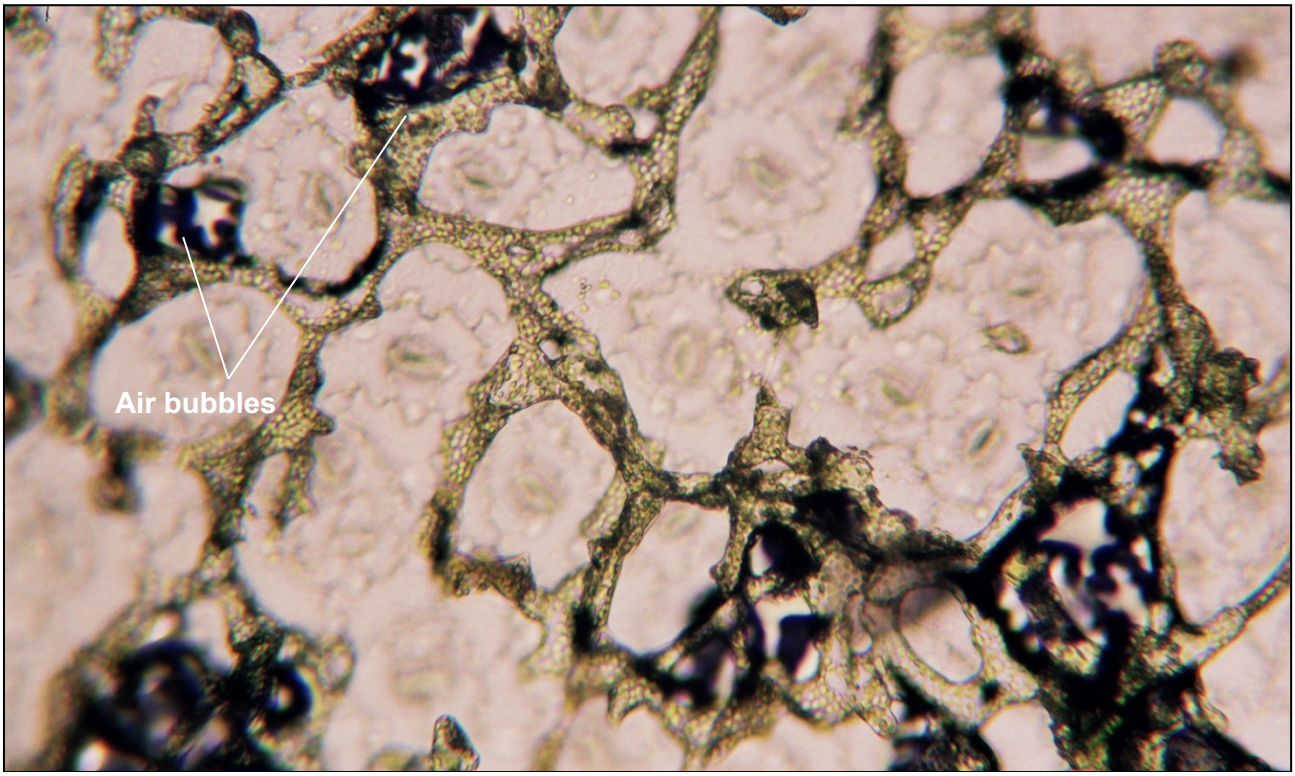
Rhubarb lower epidermal tear:
Low Power (x40) above, Medium Power (x100) below



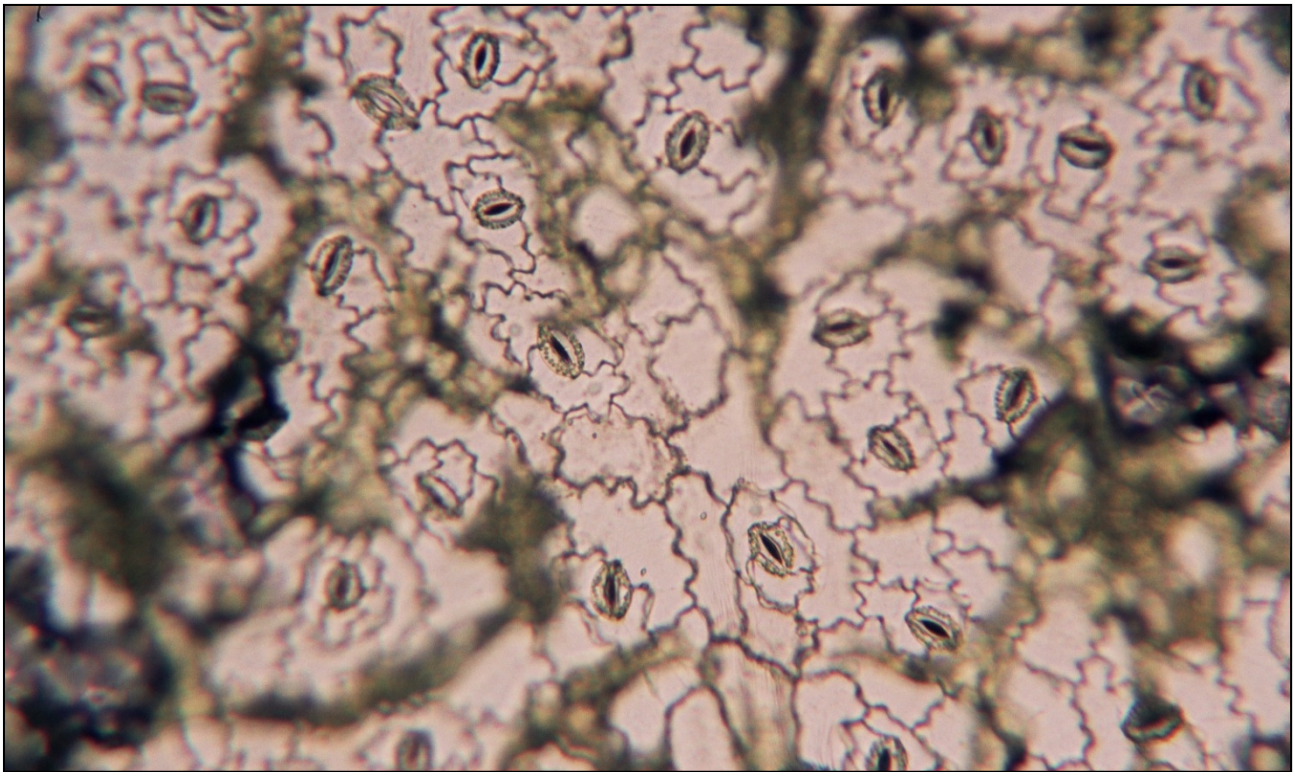
Note that in both these photographs some of the spongy mesophyll cells have adhered to the epidermal layer. This occurs commonly and they can be ignored. Often they will be out of focus as they will be at a different level to the epidermal layer. This phenomenon is explored further in the next two photographs.

In these photographs, the epidermal tear is mounted up-side-down so that any spongy cells will be on top and thus more easily seen. The photographs were taken with the microscope focused at different levels:

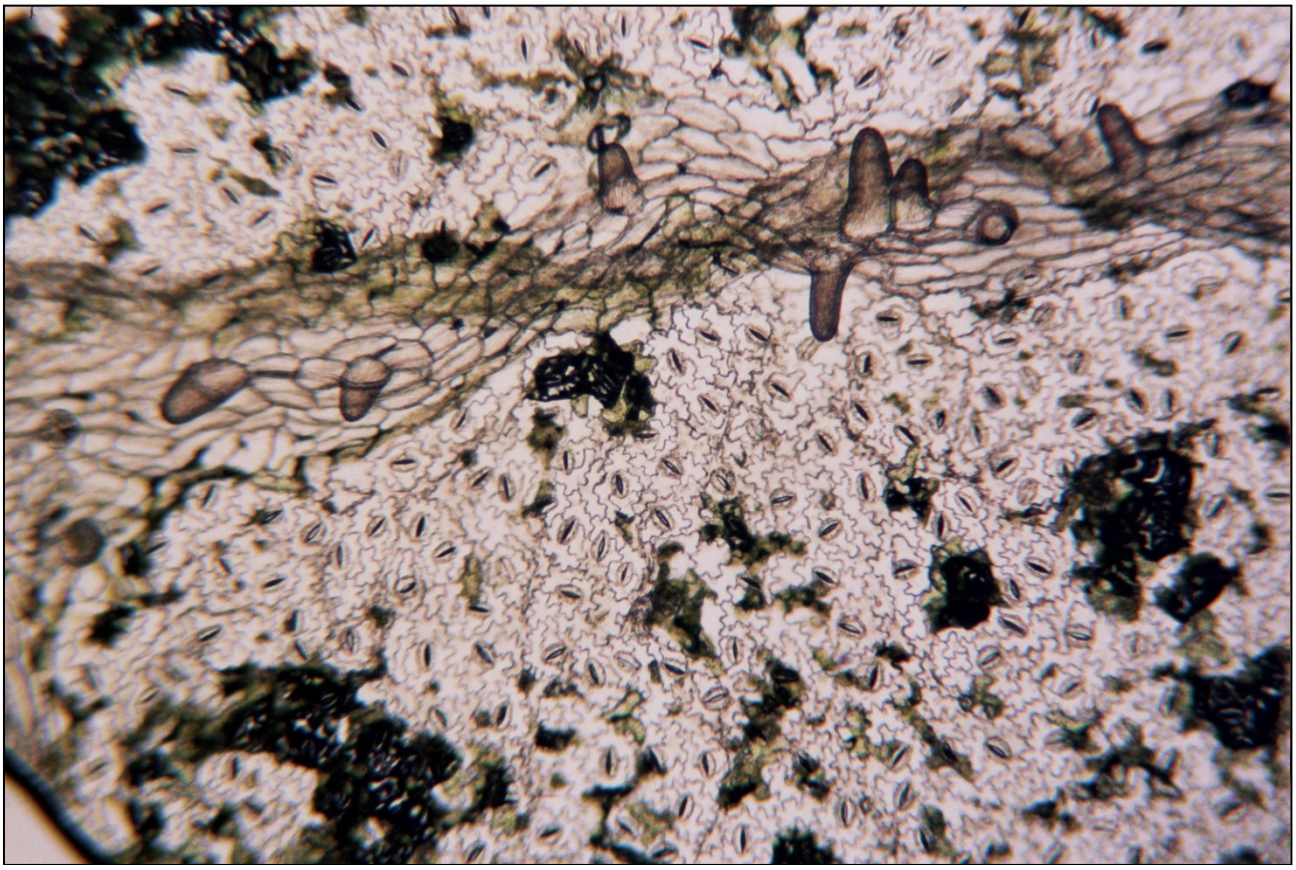
- focused on the spongy cells (top photograph). Note their elongated and interconnecting shape that give a large surface area. There are also some air bubbles trapped between these cells.
- focused on the epidermal cells beneath (bottom photograph), the spongy cells are now out of focus.



Rhubarb Lower Epidermal tear spongy cells upwards:
Both photographs Medium Power (x100)



Another feature that can cause confusion is the different shapes of the epidermal cell that are present above and below the larger veins in many species. The following photograph illustrates this:



Rhubarb Lower Epidermal tear: Low Power (x40)

Note the following:

- Epidermal cells are normally jigsaw shaped. They have wavy outlines and fit together to form a complete, intact layer. Above and below veins (as in this photograph) their shape is different and they no longer exhibit the wavy shape. They do however still form a complete layer that protects the leaf.
- In the photograph above, there are some small epidermal hairs. These are extensions of the epidermal cells themselves and have been squashed flat by the cover slip on top of them.

Stomatal patterns in Monocotyledons

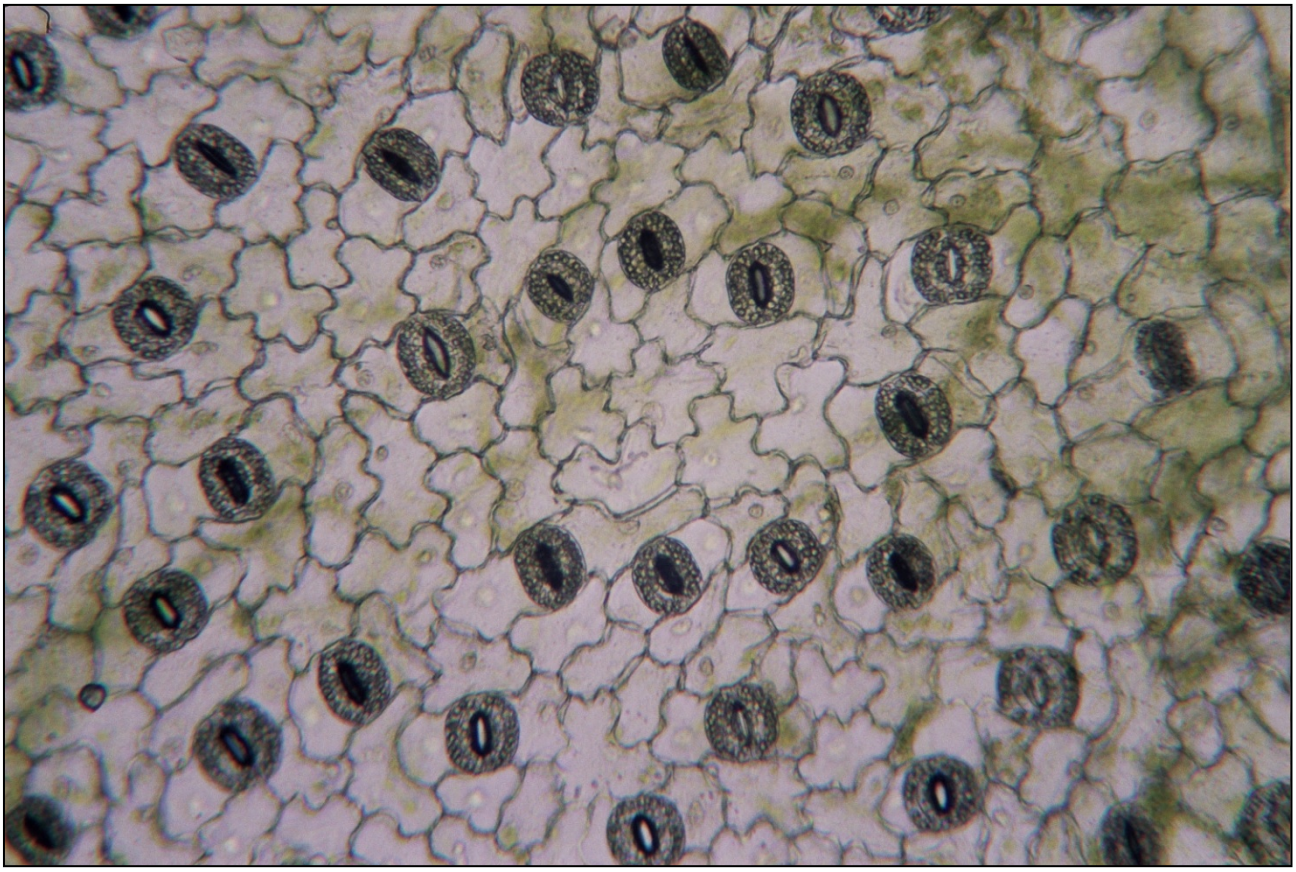
All the photographs so far have been of Rhubarb, which is a dicotyledon. Stomata are distributed in a relatively regular spacing pattern, with each stoma a minimum distance from the next one. The orientation of the stomata is random.

Monocotyledons usually have a different (more regular) pattern. Their veins are parallel rather than the net venation pattern seen in dicotyledons. Their stomata also show a more regular orientation as shown in the photographs on the next page:

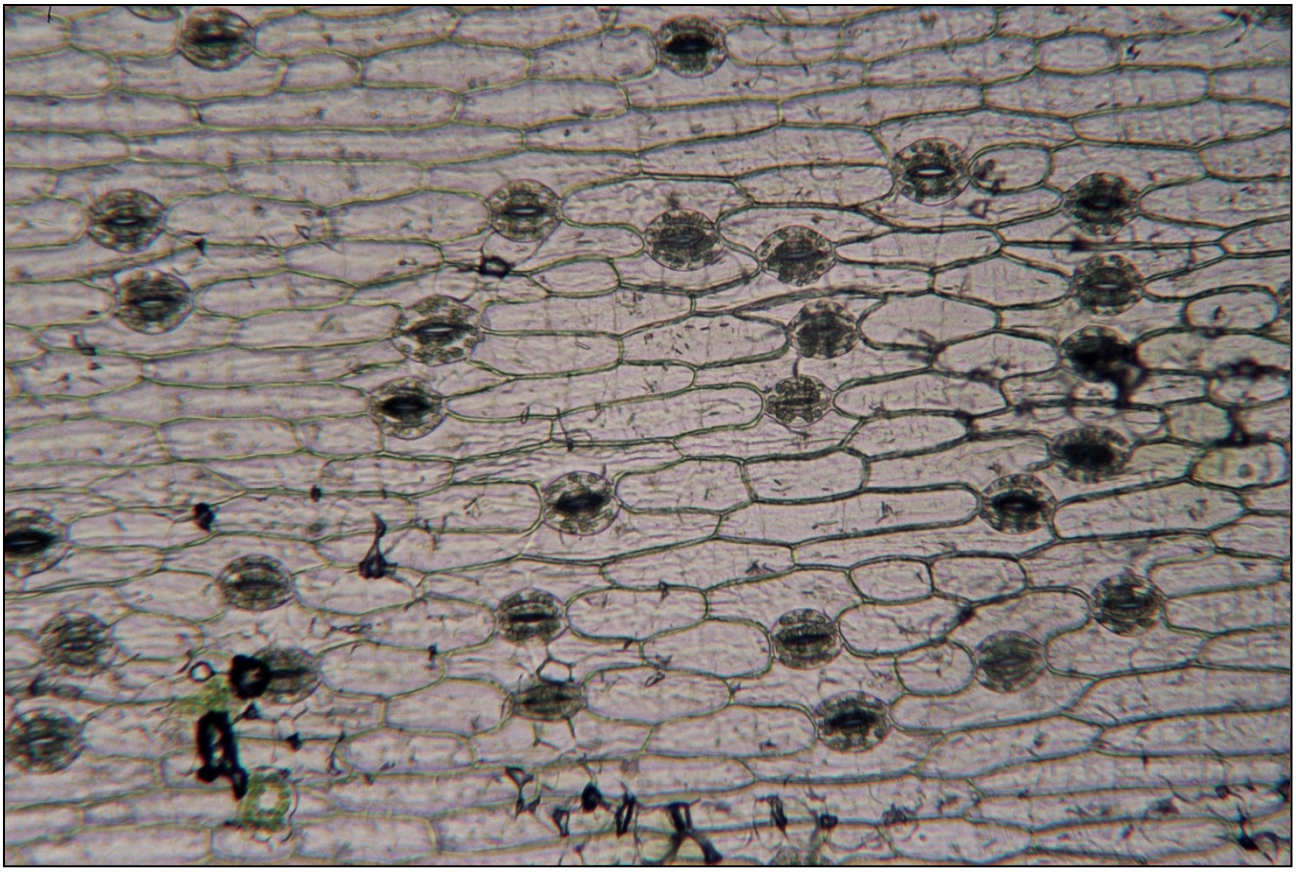
Note the following:

- The epidermal cells are often elongated and oriented along the length of the leaf i.e. their long axis is the same as the long axis of the veins and the strap-like leaves of most monocotyledons
- The stomata are all oriented in the same direction (again parallel to the length of the leaf) rather than in random directions as in dicotyledons.

Monocotyledon leaf tears.



Hosta Leaf tear: Medium Power (x100)

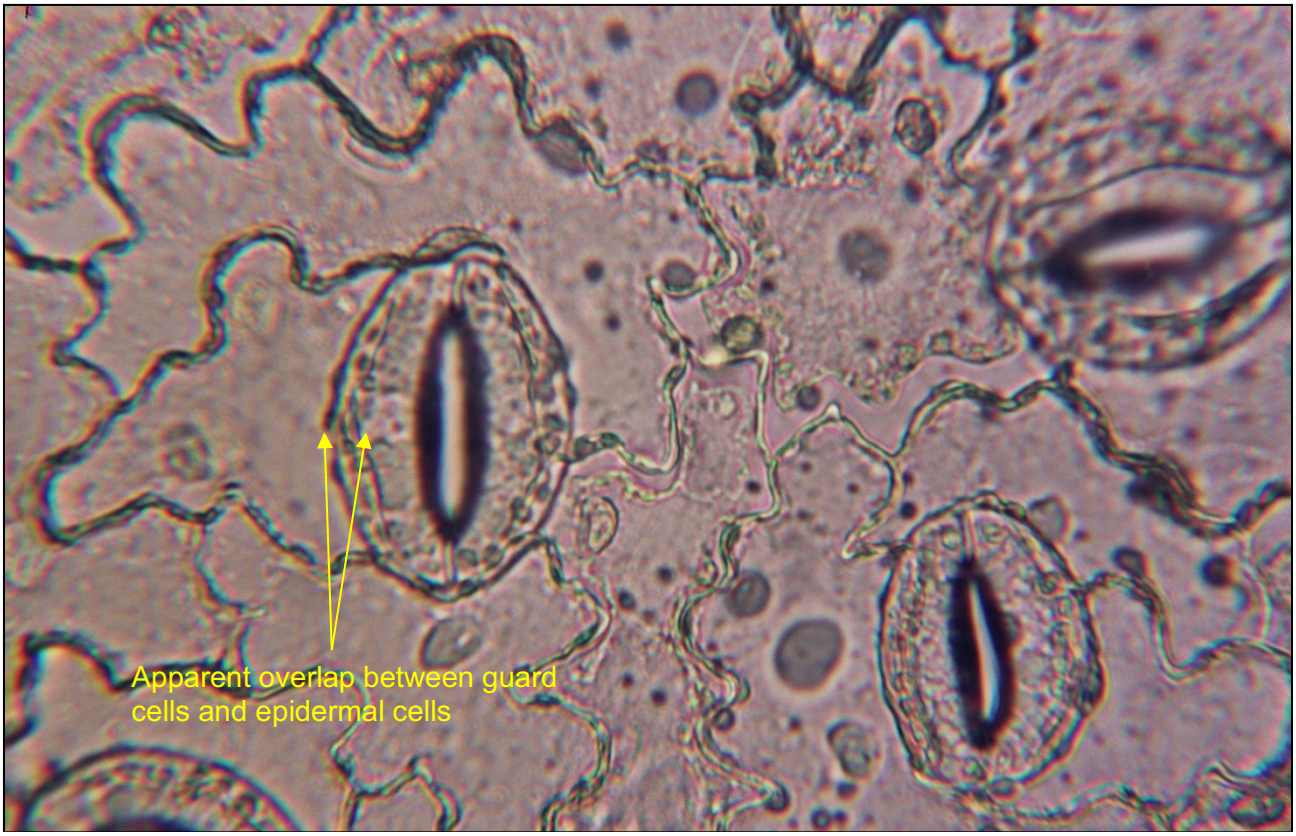


Agapanthus Leaf tear: Medium Power (x100)

Guard cells and stomata

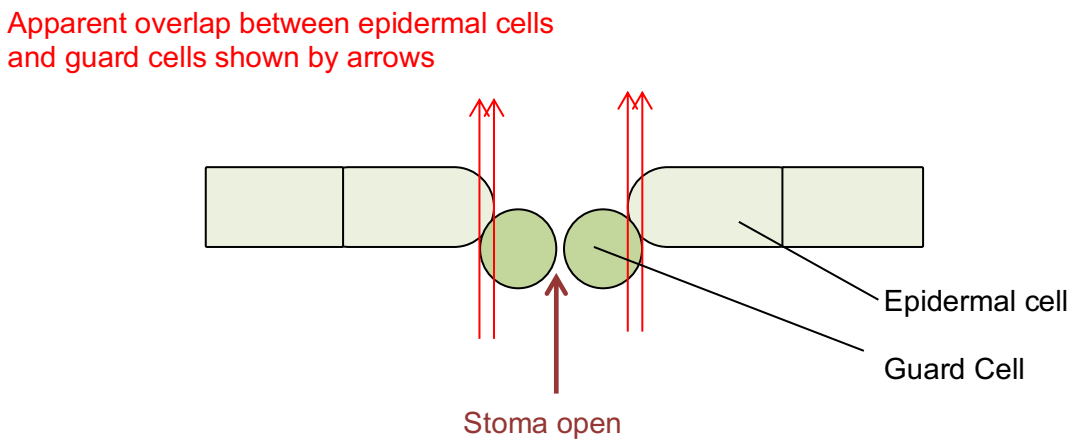
Before you try to draw what you see in a diagram, it is also necessary to first investigate the structure and function of stomata.
Study the 2 photographs that follow and the notes on the next page:

Rhubarb stomata: both photographs High Power (x400)



Features to note about the structure of guard cells and stomata:

- Guard cells are always in pairs. The stoma (plural stomata) is the pore between them.
- Guard cells have chloroplasts. This is unusual for epidermal cells which are generally colourless because they lack chloroplasts.
  The presence of chloroplasts means photosynthesis will occur. This will lead to an accumulation of sugars in daylight and the guard cells will absorb water (by osmosis) from neighbouring epidermal cells, which lack chloroplasts and thus do not accumulate sugars. The guard cells become turgid.
- The chloroplasts of guard cells are not distributed randomly. They normally occur in a row along the length of the cell.
- Guard cells have a cell wall that is not of uniform thickness. The cell wall is much thicker (and thus incapable of stretching) on the inside (next to the stoma).
  (The darkness of the inside cell wall is because the thicker wall refracts light away from the objective lens. Because no light is transmitted through this part of the cell wall to the lens the cell wall in this region appears black).
- Guard cells are curved (sausage-shaped). As they absorb water, the cells become turgid and the cell wall stretches to accommodate this. Because the internal cell wall does not stretch the outside wall expands and the cell 'bends'. When this happens to both guard cells the stoma opens.
- There is an area of overlap between the epidermal cells and the guard cells. Imagine a cross-section of the stoma, its guard cells and the adjacent epidermal cells. The following diagram illustrates this cross-section. The red arrows indicate the path of light through the specimen and up to the objective lens of the microscope. The overlap between these cells occurs because the guard cells are at a lower level than the epidermal cells (they are slightly sunken into the leaf surface).



Are stomata only found on the lower surface of a leaf?

One way to find the answer to this question is to do leaf tears of both the upper and lower Epidermal layers and observe both under the microscope.
There is a simpler method however:

- Use fresh turgid leaves
- Holding the petiole, plunge them into a 250 ml beaker of hot water. Look for small air bubbles on the leaf surface.

What happens is that the air inside the leaf is suddenly heated and expands. As the pressure of this air increases it escapes through the stomata forming small bubbles on the leaf surface as it does so.
If you see these bubbles on the upper surface them there are stomata there and this can then be verified by doing an upper Epidermal leaf tear.

Leaf Epidermal tear diagram: