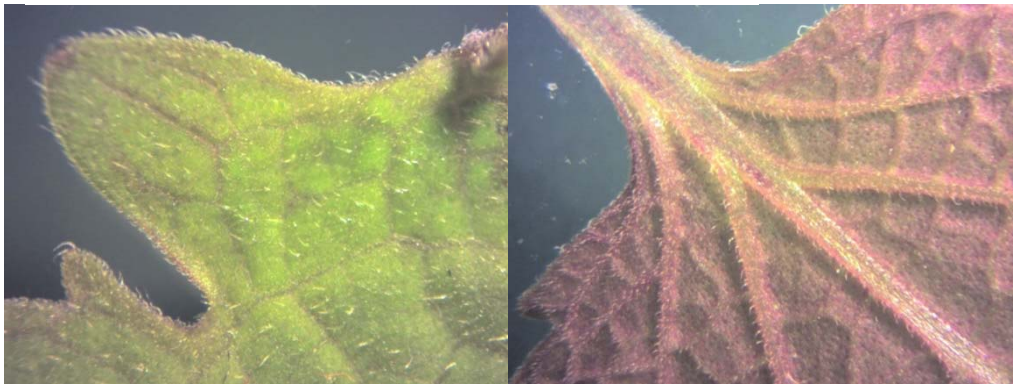
Measuring Stomatal Density

Leaf impression method

Stomata are the pores found on the surface of leaves and some stems. They are formed from two guard cells (bean-shaped cells) joined at their ends, forming a pore. Stomatal pores open and close in association with light availability and osmotic changes, allowing CO_2 to enter the plant for photosynthesis. When stomata are open O_2 diffuses out of the leaf and H_2O vapour leaves the plant by transpiration. Plants need to regulate the amount of time the pore is open to maximise CO_2 entry for photosynthesis and limit water loss. Plants adapted to different environmental conditions may have variable number and/or location of stomata on the surface of the leaves. The upper and lower surfaces of a leaf may have similar or vastly different densities of stomata.

Which surface of this leaf has most stomata?



The location and density of stomata can be determined with microscopic techniques. The method described here is the preparation of a leaf surface impression or cast, allowing for identification and quantitation of stomata.

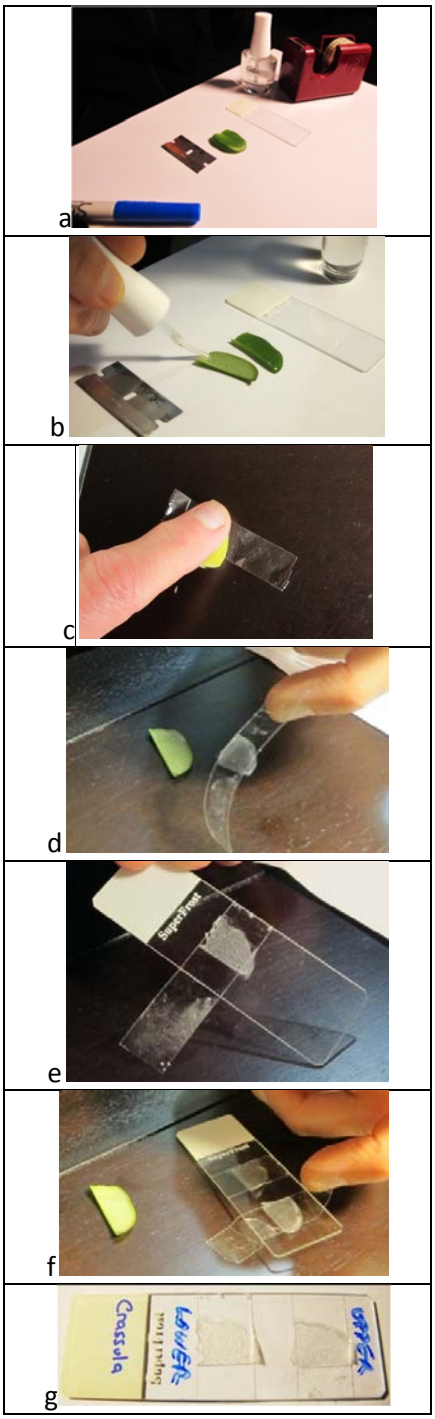
Materials

- Plants with suitable leaves. Smooth leaves without many leaf hairs are usually best
- Compound microscope with magnification up to 400x
- Microscope slides
- Forceps and scalpel blades
- Clear nail polish
- Clear sticky tape
- Marker pen
- Digital camera to capture microscope images of the leaf surfaces – this greatly aids in counting stomata and is a record of the results for future reference

Method - impression of leaf surface to quantify stomata

This method uses clear nail polish to make an impression or cast of the leaf surface. The cast is removed with sticky tape and placed on a microscope slide.

a) Collect materials. Find a suitable leaf. Identify the upper and lower surfaces of the leaf, as they are on the plant under normal conditions.
b) Spread a thin layer of clear nail polish on EACH SURFACE, upper side and lower side, the leaf surface. Leave it to dry. (it may help to cut the leaf and coat an upper and a lower surface at the same time)
c) Place a strip of clear stick tape over the nail polish. **Press** the tape down to make a good connection with the nail polish.
d) Peel off the sticky tape; the layer of nail polish should come off with the tape.
e) Place the tape with leaf impression on a microscope slide. Use a razor blade to trim the excess sticky tape from the edge of the slide.
f) Place the impression from the other side of the leaf on the other part of the slide.
g) Label the slide with the plant name, and the upper and lower surface.
h) View under the microscope at 100x or 400x magnification.

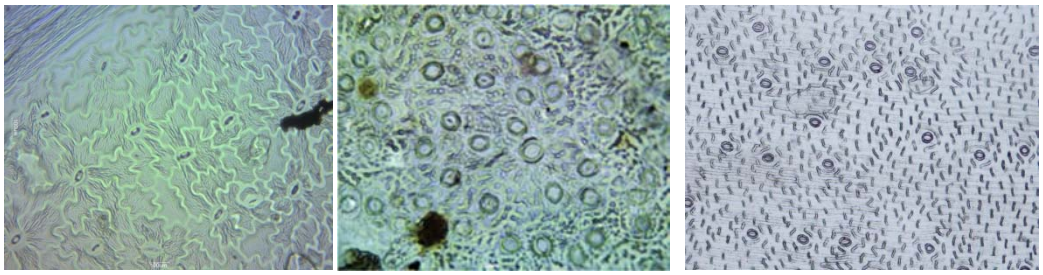


Observations and recording results

i) Make sure that you can identify the stomata compared to epidermal cells.
j) Choose a magnification that gives a number of stomata you can keep track of when counting – too many cannot be accurately counted.
k) Decide on rules for counting (e.g. the whole stoma must be in view to be counted)
l) Count the stomata in at least 3 FOVs on each leaf surface. Record the results in a table and calculate stomatal density.

Keep in mind that the size and shape of the epidermal cells and stomata varies in different species.

Figure below: Examples of leaf impressions from three different plants, showing variety in cell size and shape.



Results

Example results table

Sample name	Magnification (ocular x objective)	Surface (upper/lower)	FOV #	Number of stomata in entire FOV	*Stomatal density **stomata/mm²**
Parsley leaf	100x	upper	1		
			2		
			3		
	100x	lower	1		
			2		
			3		

* using previous microscope calibration values. As a rough guide, the area of the FOV at 100x magnification is around 1800 µm (1.8mm). The area of the FOV at 400x is around 450µm (0.45mm).

Area of FOV = πr^2

Stomatal density = number of stomata in entire FOV / area (mm^2)

Advantages and limitations of the impression method for counting stomata, compared to epidermal peel.

Advantages of the impression method:

- More reliable as larger surface area is obtained and more fields of view can be counted
- Quick and easy with smooth leaves

Limitations of the impression method:

- Leaf hairs limit ability to get nail polish onto leaf
- Leaf hairs and other irregularities in the leaf surface cause 'air bubbles' that interfere with viewing
- Sunken stomata may be difficult to identify or interpret
- Organelles are not seen – so chloroplasts cannot be seen to help identification of guard cells– this can make it difficult to interpret open vs closed stomata
- Nail polish solvent may damage leaf

Possible Investigations using the methods described

Some potential investigations that could be conducted by students with the methods described.

Stomata Location and Density:

- presence of stomata on upper, lower or both sides of leaves
- density of stomata on each side of leaf
- monocotyledons vs dicotyledons
- different Genus related to environment: succulents vs cool climate plants
- variation in density over the leaf surface
- variation on different leaves of the same plant: young vs old leaves, young vs mature plant
- variation on leaves of plants from the same genus (e.g. *Brassicaceae* – brussels sprouts, cabbage, broccoli, etc.)

Stomata opening: (interpreting open and closed stomata is harder – may give unreliable or invalid data)

- time of day/light conditions (artificial vs sunlight)
- water availability
- CO_2 availability

Experimental Design: Controls and Variables

Consider the following points.

a. Recording the results: number of FOV to count, rules for counting

b. Calculating the density of stomata

- Calibrate the microscope so that you know the size of FOV, and can calculate area = πr^2
- Record magnification with each viewing
- Use magnification that allows accurate counting – images make the task more accurate
- Keeping track of which side of the leaf is viewed
- Data collection spreadsheet
- Count at least 3 FOV per sample
- Calculate stomatal density (stomata/mm^2)

References

Science and Plants for Schools http://www.saps.org.uk/secondary/teaching-resources/299-measuring-stomatal-density

Nuffield Foundation http://www.nuffieldfoundation.org/practical-biology/window-past-measuring-stomatal-density

Measuring Stomatal Density using nail varnish
http://www.rothamsted.ac.uk/sites/default/files/groups/bioimaging_dev/Measuring%20Stomatal%20Density.pdf