In this exercise we will consider the internal structures of the foliage leaves from several species of flowering plant leaves. We will be studying the three main tissue types in the leaf: the epidermis, the mesophyll layers (two types) and the vascular system. When you look at the vascular bundles within a leaf, think about where (which side) you find the xylem and phloem and how this matches up with the positioning of the xylem and phloem in the young stem where the leaf was attached. You should be able to tell “which way is up” for a leaf section based on this consideration.

Hand sectioning of leaves

Most of the cells you will look at have fairly thin walls (with the exception of some of the cells associated with vascular bundles). This means that your sections must be thin and clean. Try a technique called the two-blade method. There are various ways of doing this and you are free to experiment. One suggested method involves inserting a thin sheet in between the two blades before cutting the leaf. Be patient and keep trying, eventually you will get a good section. Thick sections will not show you what you need to see and even worse; they will tend to lie on the microscope slide with the epidermis up instead of with the leaf on its side.

Stain with toluidine blue, but only for 15 to 30 seconds. You just want to lightly stain the epidermis, the vascular tissue, and the walls of exposed cells. If the section is too dark, it will be hard to see chloroplasts in the mesophyll cells.

Typical dicot leaves
Try the following observations with both English ivy and mulberry leaves. Make sure you see good views of at least one of these species. They will both show typical dicot leaf structures, although they are not identical.

- **Epidermis:** Observe the epidermal layer on both sides of the leaf. Which has guard cells?
- **Mesophyll – palisade:** Find the palisade layer(s). Note the shape of these cells (compared to the other mesophyll cell type). How many layers are there?
- **Mesophyll – spongy:** Find the spongy mesophyll cells. Note their shapes. Compare mesophyll cell types in terms of:
  - intercellular air spaces
  - number of chloroplasts in each cell
- **Observe the vascular bundles that pass through the leaf.** Note that they occur in various sizes (numbers of xylem and phloem cells, diameter of the cells).
  - The bundles of some species are partially or completely surrounded by sclerenchyma fibers. Make sure you can distinguish these cells from phloem sieve elements and xylem cells.
  - Make sure that you can distinguish the xylem vessels from the phloem sieve elements. The effect of the toluidine blue stain and the thickness of the cell walls should help.
  - Also make sure that you understand the relationship between the position of the xylem and phloem and which side of the leaf is adaxial or abaxial.
- **Position and orientation aspects of leaf structure.** You should be able to look at any leaf section and tell:
  - adaxial vs. abaxial
  - which surface contain stomata
  - which surface was “up” (palisade usually on the sunlit side)

**Typical monocot leaves**

We will look at corn leaves in part II (next week) because they also show the anatomy specific to C₄ plants (Kranz anatomy).

**Yucca brevifolia** (Joshua tree)

- Prepare a few thin sections of the yucca leaf. The leaf is thick enough so that you do not need to use the two-blade method.
- Observe the thick cuticle outside of the epidermis. Find guard cells and note the substomatal cavity (air space) below the stomatal pore.
- Observe the mesophyll tissues. Are there both palisade and spongy mesophyll cell layers? How many palisade layers are present? Think of a relationship between the numbers of palisade layers and the light
environment for this and other species we will look at in these two labs on leaves.

- Observe the massive amounts of water storage tissue. What kind of cells are these? Chloroplasts? Think about their wall thickness in terms of the ability to shrink and swell as they provide water for other tissues.

- This species is an example of one having a unifacial leaf. What does this mean? (The leaves we looked at earlier were bifacial.) Look at the vascular bundles. On which side do you find the xylem and phloem for bundles on different sides of the leaf? Now think about why leaves and stems are not as far apart as might seem. Also note the similarity between leaf sides in terms of the palisade layers.

- Locate the vascular bundles and identify phloem sieve elements, xylem vessels, and sclerenchyma fibers.