Introduction: Cells are the fundamental units of life, and nothing simpler than a cell is actually alive. Plant cells, like animal cells (and unlike bacteria), are eukaryotic. Eukaryotic cells have nuclei and structures called organelles where different biological processes occur. The entire eukaryotic cell is pervaded by a membrane system called the endoplasmic reticulum. Plant cells, unlike animal cells, have a cell wall and plastids. In this exercise, you will observe examples of relatively undifferentiated plant cells. During the exercise you will be asked either to label figures of the cells you observe, or to draw those cells. Lots of time has been allocated to these tasks, so take your time and make careful observations.

I. The Elodea leaf cell: a photosynthetic factory.

Procedure: Make a wet mount of a leaf of Elodea. Plants are at your bench and you simply need to pluck a leaf off the shoot.

Observe the leaf with your scanning objective (40x total magnification). Even at low power, it will be obvious that most of these cells are full of chloroplasts.

Chloroplasts. These are green, membrane-bound structures that are the site of photosynthesis. Chloroplasts belong to a class of organelles called plastids. Plastids are thought to have been derived from free living photosynthetic bacteria that were engulfed by a unicellular organism. This joined organism gave rise to algae which evolved into plants. Evidence for the idea include the presence of DNA in plastids. This DNA is arranged in rings like that of bacteria. Plastids also have ribosomes (the structures that are the site of protein synthesis). These ribosomes are different from those found in the rest of the plant cell, and are like those of bacteria. Plastids are surrounded by a double membrane system. The inner membrane is thought to have been derived from the bacterium, and the outer one from the host cell. Chloroplasts make vegetation green.

The gross structure of the Elodea leaf and cells: Switch to high power (400x) and carefully through-focus.

1. How many cell layers thick is this tissue? ________________

Continue through-focusing but carefully study one cell.

2. Based on your observations, are the chloroplasts evenly distributed throughout the cell?
   __________________________________________________________________________
   __________________________________________________________________________
   __________________________________________________________________________
3. How are they distributed?

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4. Carefully observe the profiles of the different chloroplasts in your cell. Given that the chloroplasts are all the same shape, how would you describe that shape?

___________________________________

Switch back to a lower magnification and look for cells manifesting any internal movement. What you are looking for isn’t subtle. Keep scanning the cells in your leaf until you find one demonstrating an obvious flowing movement of the chloroplasts around the periphery of the cell. This movement is called cyclosis or cytoplasmic streaming. It is facilitated by the same two proteins responsible for muscle contraction in animals (actin and myosin).

While observing a streaming strand of cytosol, look carefully for spherical structures about 5% of the diameter of the disc of the chloroplasts. These are the mitochondria.

Locate a nucleus (if you haven’t already). Often they will be found against one side of the cell in which case they will be hemispherical in outline. If you have difficulty finding one ask your TA for help (but search for a good five minutes first). Can you see a nucleolus in your nucleus?

**Drawings:**

a. Make two drawings of the same cell: one with the focus just below the cell wall and the second with the focus at the midpoint of the cell. Label chloroplasts in each view: note their different orientations. In the second view, the area in the center without chloroplasts is occupied by a large central vacuole. This structure contains a solution called cell sap, and can occupy up to 90% of the volume of the cell. Label the vacuole in your drawing. If you look closely while through-focusing, you will observe strands of cytosol transversing the vacuole. Include these in your second drawing. If you were successful in observing mitochondria include these in your drawing also.
b. If a nucleus was not included in the drawings for “b” make a third drawing with a nucleus: label the nucleolus if you see it clearly.

Based on your observations, why are vegetables such as lettuce and cabbage low in calories? Assume that their cells are similar to those of *Elodea*.

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II. The *Tradescantia* Stamen Hair, Plant Cells Without Chloroplasts.

In *Elodea* cells, chloroplasts interfere with the view of the over-all structure of the plant cell. In this activity, you will view plant cells without chloroplasts.

**Procedure:** The cells you wish to observe are associated with the male parts of the *Tradescantia* flower (the stamen). The stamen has two parts, the anther containing the pollen, and a supporting structure called the filament. The filaments in *Tradescantia* are hairy, and these hairs are what we wish to view. Remove an entire stamen with your forceps and place in a drop of water on a slide.
With your teasing needles, slice off the anther, and carefully lower a cover slip over the filament. Many of the hairs will trap air. Ignore those parts of the slide with entrapped air. Carefully view a living cell totally emersed in water at high power. Your cell may contain a water soluble pigment in the vacuole. If so, discerning the boundary of the vacuole will be obvious. If your cell does not have pigment dissolved in the cell sap, this boundary can be identified as the point of transition between the granular cytosol and the clear solution in the vacuole. Identify cytostolic strands transversing the vacuole. Note any cytoplasmic streaming, and the prominent nucleus. In these cells the nucleus is often suspended in an island of cytosol surrounded by the vacuole. This island is connected to the sheets of cytosol just below the cell wall by the cytoplasmic strands that cut across the vacuole.

Label figure E-1 below

![Figure E-1](image)

III. Other Plastids

**IIIa1. Chromoplasts in Flower Petals:**

Tear a piece of tissue from an *Allamanda* flower and make a wet mount. Carefully view the intact margin at 400x. Through-focus and observe the yellow translucent chromoplasts.
Draw a petal cell - label the chromoplasts

IIIa2. Water-soluble pigments in *Impatiens* flower petals:

Many flowers are not colored by chromoplasts. Prepare a wet mount of *Impatiens* flower petal tissue using the same procedure used for *Allamanda*. Again observe an intact margin and through-focus.

6. What part of the cell is colored? _______________________________

Draw a cell from the intact margin and label the boundary of the vacuole, and label the vacuole, itself.

IIIb. Chromoplasts in Fruit

Plastids can be transformed into other forms of plastids. As some fruits ripen, chloroplasts are transformed into chromoplasts. The resulting color change serves as a visual signal to animals that the fruit is ripe. This ensures that the seeds inside the fruit will not be dispersed until they are mature.

Make wet mounts of thin sections of green unripe and red ripe pepper fruit.
Make drawings of each tissue: label chloroplasts and chromoplasts.

IIIc. Pigment Bodies in Carrot

The pigments in chromoplasts are fat soluble and can often be observed as discrete droplets or crystals. These structures are **pigment bodies**. In some tissues the chromoplasts become disrupted and pigment bodies are found directly in the cytosol. This is the case in carrot tissue. Carotene pigment (the source of vitamin A in the tissue), is produced in chromoplasts and becomes incorporated into a long narrow crystal twisted and under tension inside the plastid. Eventually these chromoplasts burst releasing the crystals which become straight. Make a wet mount of carrot tissue and observe these pigment bodies. If you see an intact chromoplast, call it to the attention of your TA.

**Draw pigment bodies in this tissue -- include a chromoplast if possible.**
IIId. Leucoplasts in *Zebrina*

Leucoplasts are colorless plastids. These may be readily observed in the intact margin of *Zebrina* leaves.

Cut a sliver of tissue from the edge of a *Zebrina* leaf and prepare a wet mount. Focus with high power on the intact leaf margin. These cells have a water soluble pigment (anthocyanin) dissolved in their vacuole. Note that the nucleus appears to be suspended in the vacuole. To understand the structure here, think back to your observations of the *Tradescantia* stamen hair and its nucleus. The structures surrounding the nucleus (making it appear bumpy) are leucoplasts.

**Draw a cell along an intact margin: label nucleus, vacuole and leucoplasts.**

IIIe. Starch Grains in Potato Tuber

All plants store starch in plastids. In some cases, plastids become so full of starch that there appears to be nothing else present except a huge starch grain. This is the case with the storage leucoplasts in the potato tissue.

Prepare a wet mount of potato. Observe the starch grains inside the cells of this tissue. Set your microscope up to observe these grains through crossed polaroids (ask your TA to demonstrate how). Note, that through crossed polaroids, the grains glow in front of a dark background. This is a characteristic of starch grains and also of crystals. **Make drawings on the next page.**

Recall the iodine test for starch used in the last topic. Please repeat this exercise here by adding I$_2$KI to your wet mount. **Make a third drawing as outlined on the next page.**
Drawings:

Diameter fov = _________ mm

Starch Grains Stained with I$_2$KI Through Crosses Polaroids

IV. The Cell Wall

In plant tissues, adjacent cells have a boundary between their walls called a **middle lamella**, and adjacent protoplasts are connected together by membrane-bound cytoplasmic channels that transverse their walls. These channels are called **plasmodesmata**. Plasmodesmata facilitate the transport of materials between cells. These two features are usually difficult to see through the light microscope. However, in the food storage tissue (the endosperm) of persimmon (**Diospyros**) seeds, these features are readily visible because the walls are huge. Take a prepared slide of **Diospyros** endosperm and observe the tissue.
at 400x. Identify the middle lamella (the lines running so as not to intersect with either protoplast) and plasmodesmata (the fine black striations running from one protoplast to another). **Label Figure E-2.**

![Figure E-2](image)

A = ____________________________________

B = ____________________________________

**V. Crystals in Plant Cells**

Crystals of calcium oxalate (a salt of oxalic acid) are frequently found in plant tissues. How they function to enhance the survival of plants is not always clear, though in some cases they may protect the plant from herbivores. In all cases, these crystals form in the vacuole. They take on different shapes in different tissues even though they are composed of the same chemical. In this exercise we will observe two types of crystals: **raphides** (needle-shaped) and **druses** (jagged spheres).

**Druses:** These are common in the tissues of pot herbs such as mustard greens, spinach, Swiss chard and collard greens. While all of these are nutritious, all of these foods increase our risk of forming kidney stones. Druses can be easily observed in the petioles of *Begonia.*
Make a thin section through the petiole of a *Begonia*. These crystals may be found in cells positioned just below the epidermis.

**Drawing:** Draw a druse in this tissue.

**Rhaphides:** These are common in many different plant tissues, and often function in defense.

Make a longitudinal section of the leaf tissue of *Sansieveria*. Look for enlarged cells with darkened structures. Switch to high power and note that these are bundles of needle-shaped crystals (raphides). Observe these through crossed polaroids.

**Drawing:** Draw raphides in this tissue.
VI. The Plant Cell Through the Transmission Electron Microscope

The invention of the scanning electron microscope revolutionized the study of plant cells and tissues. With the electron microscope, details of cellular structure barely visible or invisible with the light microscope can be viewed with startling detail. Other structures such as ribosomes may be viewed only with the electron microscope. Below is an EM of a young plant cell:

For Figure E-3 (below), note the nucleus (N) surrounded by a double unit membrane; the cell wall (CW) with its laminated (often amorphous) structure; mitochondria (M) with their internal cristae, the vacuoles surrounded by a single membrane (tonoplast), and the endoplasmic reticulum (ER). The dots throughout are ribosomes.

![Figure E-3](image)

**Identify and label these structures on the figures that follow:**

**Nucleus:** identified by its size, double unit membrane, and granular texture (due to chromatin).

**Cell Wall:** identified by its laminated or amorphous texture.

**Mitochondria:** identified by their size, by their double unit membrane, and by the enfoldings of the inner membrane called **cristae**.
**Plastids:** Identified by their double unit membrane.

**Leucoplasts** can be identified by their absence of cristae or chromatin. Leucoplasts may have amorphous starch grains, or crystalline protein.

**Chloroplasts** can be identified by their stacks of thallakoid membranes called grana.

**Vacuole - Vacuole membrane:** Vacuoles are surrounded by a single unit membrane. The texture inside is clear - evidence of the absence of other cellular components.

**Microbodies:** Have a single unit membrane and are usually dense in appearance.

**Golgi Bodies:** In cross section appear as a stack of membrane-bound compartments resembling a cross section of a stack of pancakes.

**Endoplasmic Reticulum:** Membranes that pervade the cell, seemingly not associated with any of the structures listed above. If ribosomes are clustered along these membranes is called **rough ER**.

**Ribosomes:** dot-like structures often associated with endoplasmic reticulum.

Figure E4

A. ________________  B. ________________
Figure E-5

A ___________  B ___________  C ___________
D ___________  E ___________

Figure E-6

A ___________  B ___________  C ___________
D ___________