**Introduction:**
The Cabrillo College Compound Microscopes:

Illustrated here is one style of compound microscopes in our Biology Laboratories. During this class you may work on another model, but it will still be very similar.

![Diagram of a compound microscope](image)

**Fig. 1**

A compound microscope (Fig. 1) has two or more objective lenses. It is used for examination of small or thinly sliced sections and provides a maximum magnification of 1000X. Illumination is from below, and the light passes through clear portions of the slide, but not through opaque regions. In order to improve contrast, sections are often prepared with opaque stains or dyes that bind to cellular structures.

**A. Parts of the Compound Microscope**

1. The LIGHT SOURCE is built into the base under the stage and is controlled by both an OFF/ON power switch and a voltage control dial that regulates light intensity (light intensity knob). The dial ranges from 0 to 10. You increase the light intensity as you turn it counterclockwise. Beginning microscopists tend to illuminate the specimen with excessive light, which eliminates what little color or contrast the specimen possesses. Therefore, develop a habit of continually varying the light levels in order to find the best level for each slide.
2. The **CONDENSER** contains a system of lenses that focuses light on the specimen. It is a structure mounted beneath the stage that condenses or narrows the beam of light and directs the light through the slide specimen. The condenser adjustment knob moves the condenser vertically. For most routine microscopy, the condenser should be in the uppermost position.

3. The **IRIS DIAPHRAGM** is mounted immediately below the condenser. Locate the lever used to regulate the iris diaphragm. Adjusting the size of the opening regulates the amount of light that can pass into the condenser. The main function of the iris diaphragm is to maximize resolution and image contrast by properly channeling the light rays passing through the specimen. With each new slide, the diaphragm will need to be readjusted. Play with the lever to see what setting works best for the slide you are viewing. (When viewing unstained specimens it is desirable to reduce the opening of the diaphragm.)

4. **OBJECTIVE LENSES** are mounted on a revolving nosepiece. Our compound microscopes have four objective lenses: low magnification (4X), medium magnification (10X), high magnification (40X) and oil immersion (100X). This highest magnification is the ONLY one to use oil. The power magnification of the lens is indicated on the side of the objective. The red is 4X, yellow is 10X, the blue is 40X and the white is 100X. The **numerical aperture** (N.A.) is also engraved on each objective. Generally, the higher the value of the numerical aperture, the better the resolving power, or resolution (that is, the ability to see as two distinct objects that are very close together). As the magnification increases, so does the resolving power (but not in a linear manner). The human eye can resolve objects about 100 µm apart, but the compound microscope has a resolution of 0.2 µm under ideal conditions. Objects closer than 0.2 µm are seen as a single fused image.

5. The **EYEPIECES** are the lenses next to your eyes as you observe the specimen. The magnification of these lenses is 10X. The total magnification of any specimen being viewed is equal to the power of the eyepieces multiplied by the power of the objective lens. For example, if the eyepiece magnifies 10X and the objective lens magnifies 40X, the total magnification is 400X (10 X 40). It is necessary to adjust binocular microscopes so that the two eyepieces form a single image. Do this by adjusting the distance between the eyepieces so that it is the same as the distance between your pupils. Move the oculars apart and look into the microscope. If two images are visible, slowly move the body tubes closer together until a single circle, the field of view, is seen with both eyes open. Be sure to find this correct adjustment for your eyes. Don’t fall into the trap of using only one eye and closing the other one. It is also necessary to correct for visual acuity differences in your own eyes. If your vision is better in one eye than the other,
you need to focus your ocular lenses individually. To make this adjustment use the 4X objective first. Look at the image through the right eyepiece with your right eye, and obtain a sharp focus on the specimen first with the coarse, then the fine adjustment knobs. Next, looking at the image with your left eyepiece, rotate the dioptr adjustment ring (found at base of left ocular) to focus on the specimen without using the coarse or fine focus adjustment knobs. When the image is clear, you have adjusted the ocular to your own eyesight.

6. The MECHANICAL STAGE (platform) is the horizontal shelf under the objectives that supports a glass specimen slide. A spring clip will hold your slide in place preventing it from shifting. Two adjustment knobs will allow you to move the slide around the stage’s platform. The upper knob moves the slide horizontally (x-axis), and the lower knob moves it vertically (y-axis).

7. The COARSE (GROSS) FOCUS ADJUSTMENT knob is used for initial focusing at low power. It should be used ONLY at low magnification (4X). The coarse focus knob is the larger of the two focus knobs.

8. The FINE FOCUS ADJUSTMENT knob is the smaller of the two focus knobs. The fine focus knob is used at all magnifications and is the ONLY focusing knob used in magnifications greater than low power. Remember: compound microscopes are par-focal which means you only need to do minimal focus adjustments when you change power. If you are turning the fine focus many times and your image is not coming into focus, something is wrong. Ask for help.

9. The POINTER is the black line that appears across the field of view in one of the ocular lenses. This line enables you to point out to a second individual particular objects or regions of the specimen.

10. The BODY TUBE is the tube that supports the ocular lenses and extends down to the nosepiece.

11. The ARM is the supportive frame of the microscope. It joins the body tube to the base.

12. The BASE is the broad, flat, lower support of the microscope that contains the light source.

B. Rules for Microscope Protection
1. The instructor will demonstrate the correct way to remove the microscope from the cabinet and how to return it. Notice that each piece of equipment is numbered and should be returned to the correct numbered location in the cabinet.

2. Always carry the scope in an upright position with the arm grasped by one hand and the base resting on the other. The eyepieces (oculars) are not attached and may fall out if the microscope is slanted or turned upside down.

3. Before returning the microscope to the cabinet, be certain that a) you have removed the last slide b) that the lowest power objective is in place over the stage opening and c) that the stage is in its lowest position. The rheostat (light intensity knob) should be turned down, and the power should be turned off.

4. Clean lenses ONLY with lens paper provided by the instructor. NEVER use handkerchiefs, Kleenex tissues, your T-shirt or Kimwipes. These materials will scratch the lenses. If the lenses are very dirty, you may moisten the lens paper with distilled water or acetone.

5. Always begin the examination of material with the lowest power objective (4X), NEVER with a high power (40X) or oil immersion (100X) objective. Compound microscopes are parfocal, that is, once an object is in focus with the low power objective, you should be able to switch to the next higher power objective without changing the focus or with a very slight adjustment in the fine focus.

6. Never use the coarse focus knob to focus downward with the high power objective or oil immersion objective in place. Use the fine focus ONLY.

7. Always cover moist, living or preserved materials with a coverslip unless otherwise directed by the instructor. Be sure to maintain a safe distance between the coverslip and the objective in order to avoid damaging the lenses.
Summary of Basic Steps for Compound Microscope Focusing

1. Rotate the nosepiece to low power, then lower stage with coarse adjustment.
2. Clip slide in place with swing arm, and center slide on stage.
3. Raise stage with coarse adjustment knob watching working distance.
4. Look through and adjust eyepiece while focusing down with coarse adjustment knob.
5. Move to medium power, use fine focus only.
6. Move to high power, use fine focus only.

C. Working with Prepared Slides

• Specimen orientation: Prepared Slide of Newsprint or Letter “e”

1. Clean the ocular, (4X, 10X and 40X) objectives and substage condenser with the special lens paper provided.

2. Plug in your microscope. Turn to and increase the light intensity dial up to about 5.

3. Make sure the stage is lowered. If the low power objective is not in position over the stage opening, rotate the nosepiece until it is.

4. Set the condenser in its uppermost position.

5. Pick up the prepared slide of the letter “e” and place it on the stage of your scope. Place the spring-loaded arm of the stage over your slide and secure it in position. Adjust the stage so that the “e” is directly over the center of the substage condenser.

6. While looking from the side, raise the stage by turning the coarse adjustment knob until it stops. While looking through the eyepiece, slowly lower the stage by turning the coarse adjustment knob until the letter “e’” comes into focus. Obtain a sharp focus by using the fine adjustment knob.

7. Adjust the eyepiece with both hands. The distance between the oculars should be such that you can comfortably see one image only with both your eyes open.

8. Adjust the diopter for your left eye.

9. Adjust the iris diaphragm to produce optimum contrast and illumination.
10. Notice the position of the ‘e’ in your field of view. Move the slide from left to right and right to left. Notice what direction it appears to be moving. Notice also that the lenses invert and reverse the orientation of the object.

11. Since microscopes are constructed to be par-focal, your slide should be at or near the correct focus point with the other objective lenses. Go to the 10X power objective now and focus only with the fine focus.

12. When finished, rotate nosepiece to 4X objective, lower the stage, remove the slide and return the slide to its proper tray.

- Practice with Through-Focusing

*Prepared Slide of Colored Threads*

1. Obtain a slide with three differently colored overlapping threads. Rotate the nosepiece so that the low power objective (4X) is in place. Focus on the threads at low power and find the point where the threads cross.

2. Check the distance between the objective lens and the slide. This is the working distance. The working distance decreases with the higher power objectives, as seen in figure 2.

Fig. 2
3. Using *only* the fine adjustment knob, slowly focus up and down through the overlapping threads, first with the 4X objective, then with the 10X objective. Notice what happens to the field of view as the magnification increases. Do not use the oil immersion until instructed to do so.

4. Notice that when you focus up and down some threads appear to be in focus when others are not. The threads do not all lie in one plane and the thickness of individual threads varies. The depth to which your microscope can focus, called the **depth of field**, is limited. The higher the magnification, the shallower the depth of field. You can study thick objects by continually changing the fine focus, thereby bringing into focus different portions and planes of the specimen. You can also use this method for determining the three dimensional form of an object. You must determine which thread is the uppermost. Record the thread order on your answer sheet.

**Depth-of-Field**

The term depth-of-field refers to the vertical distance that is in focus at any one time. Higher magnification lenses have a smaller depth of field – that is, only a thin horizontal slice of your sample may be in focus at any one time. So, at high magnification, very small adjustments to the fine focus can quickly take your sample completely out of focus.

**Field of View**

The term field-of-view refers to how much of the horizontal area of your sample you can see at any one time. The field of view decreases with increased magnification. As you increase the magnification, you are looking at progressively smaller subsections of your sample. That is why you need to be sure to center what you are viewing before increasing the magnification.

**D. Wet Mount Preparation: Working with Living Cells**

- *Working with Living Cells and Tissues*
  
  *Preparation of Elodea Leaf*

Often it is necessary to prepare a specimen for viewing. In such cases, the object should always be viewed as a wet mount. This type of slide is prepared by placing a drop of liquid on a slide, or by placing the dry specimen on the slide and adding a drop of water. The mount is then covered with a coverslip as shown in figure 4 below.
1. Prepare a wet mount of the *Elodea* leaf provided. Choose a fresh leaf that is intact. When obtaining a slide and coverslip make sure you have only one and not two stuck together. Hold the coverslip at a 45% angle before dropping it onto the specimen. This reduces the appearance of huge black “organisms” better known as air bubbles.

2. Observe the leaf first with the low power and then with the medium power objective. Note the numerous green chloroplasts, the site of photosynthesis. Go to the high power objective and examine an individual cell. Using the fine focus, focus on the upper surface of the cell. Gradually focus downward through the cell to the lower surface, and then to the top surface again. What is the three dimensional shape of the cell? Go back through the cell again and continue to the lower surface. Is there another layer of cells present? The procedure you have used is known as “through focusing” and is used to determine the shape of a cell or tissue.

You will need to draw what you are seeing under the microscope.

3. Draw a cell of *Elodea* at 40X and 100X. Label the cell wall, the chloroplasts, the clear vacuole and the nucleus if you can find one. The cell wall is a porous, non-living, relatively rigid structure composed primarily of the complex polysaccharide cellulose. The cell membrane is found closely applied to the inside of the cell wall. When the cell’s vacuole contains all the
fluid it can hold, the cell appears “plump” or turgid, and the cell wall and cell membrane cannot
be distinguished because the cell membrane is pressed tightly against the cell wall.

4. Notice the cytoplasmatic streaming (often called cyclosis) seen in some cells. Carefully focus
just below the layer of chloroplasts.

5. Remove your *Elodea* leaf, dry your slide, place the *Elodea* back on the slide, and add 0.4M
sodium chloride solution to prepare a second wet mount. The concentrated salt solution will
cause water loss from the cell, and the separation of the cell membrane from the cell wall. The
phenomenon you see is called *plasmolysis*. Although you cannot see the cell membrane per se,
you can see the outer limit of the cytoplasm where the cell membrane occurs. Is plasmolysis
reversible? How could you determine this? Try your proposed method. **Dry off your slide
and cover slip and save it for the cheek slide preparation coming up in section F.**

E. **Working with **BIOHAZARD** materials!**

As an example of an animal cell, you shall view some of your very own cells from the inner
lining of your cheeks.

1. **GENTLY** scrape the inside of your cheek with a clean toothpick and smear the scrapings on a
clean, dry slide. You don’t need to poke, a gentle rubbing will dislodge cells from the epidermis
of your inner cheek. Dispose of the toothpick in the red biohazard bag.

2. You should be able to see a white, wet smear on your slide. Let the slide air dry and then
cover the dried cells with one drop of methylene blue stain. Remember this is a stain that will
stain clothes, floors and hands indiscriminately.

3. **AFTER ONE MINUTE,** allow the excess stain to drip into the supplied container. We do not
let the stain flow down the drain. Rinse off the dye by letting a small steady stream of tap water
run over your slide, avoiding the exact location of the smear. You do not want to wash off all
your cheek cells.

4. Add a cover slip, blot both the top of the slide and the bottom with a Kimwipe and examine at
low, medium and high power.
5. Notice the large, central nucleus and round shape of the cells. Draw two or three cells as viewed with the 40X or 100X power objective.

![Fig. 4. Human Cheek cells](image)

6. After viewing the slide, draw it.

You don’t need to be a great artist to draw useful diagrams. Here are some tips for producing good drawings that can help you learn the material and study for practical exams. Various Biology courses will have you drawing and you want your drawing to serve as a study tool.

a. First look at the diagram of the tissue above (Fig 4) to get oriented and to get an idea of what you should look for in the field of view.

b. Make note of the image’s magnification. As always, start on low power, then advance to the higher powered objective lenses. You should aim for a magnification similar to that in the diagram.

c. Reproduce as closely as possible what you see in the field of view using a pencil. This does not have to be a work of art, but you should take care to draw the shape of the cells, and the way the cells are organized.

d. Only add color if you wish. Studying colored images can be quite helpful. Sometimes one tissue will come in different stains, and you will want ot make a note of it.

e. The final step is to label your drawing. Use your lab manual, textbook, and other resources as a guide to ensure that your labels are accurate.

f. Add a scale to your drawing- even if it is limited to the magnification that you used. This way you can have an idea of the real size of your specimen.
7. When finally finished with the slide, put it in the “biohazard”, or red plastic bag found on the instructor’s cart or side of the room. Your slide contains body fluids and should be disposed of correctly. Please help us keep our labs clean.

It is not likely that in our labs you will encounter an exploding bomb or a material that is of acute toxicity, but please make note of the warning signs that are used globally to indicate hazards or warn us of health risks. Paying attention to labels is crucial in the lab setting.


F. Using Immersion Oil

*Prepared Slide of Human Blood Smear*

**Magnification** refers to increasing the size of an image. **Resolution** refers to the ability to distinguish two points. In the figure below, resolution decreases from left to right, even while the magnification of the image remains the same. Better resolution produces a sharper image with more detail (Figure 5).

As magnification increases, resolution decreases but this loss of clarity becomes noticeable only at very high magnification. When an object is magnified 1000X, the image is bigger but blurry. This is due to the way light bends as it passes through glass (the slide), then air, then glass (the lens). This bending of light is called **refraction**. Filling the air space with oil decreases the bending of the light, which improves resolution and produces a sharper image (Figure 6). However, oil is ONLY used with the oil immersion objective (1000X magnification).

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![Figure 5](image1.png)

Fig. 5. Images of two dots with decreasing resolution from left to right

![Figure 6](image2.png)

Fig. 6. Immersion oil improves resolution by decreasing the refraction of light
**Procedure:**

1. Focus the human blood slide under low (40X), medium (100X) and high magnification (400X) as before. No oil is used at these magnifications. Most of the cells you see will be red blood cells (RBCs), with a scattering of rarer cell types called white blood cells (WBCs). While there is only one type of RBC, there are five different types of WBCs. The different types of WBCs are cytologically distinguished by their size, nucleus morphology, and staining properties (color). See below and the reference posters provided in class for more information on the different types of WBCs.

2. Using 400X magnification, make a simple sketch of a small segment of the field of view. You do not need to sketch every cell you see.

3. **Using the Oil Immersion Lens** – Without lowering the stage or moving the focusing knobs, rotate the nosepiece so that the high power and the oil immersion lenses straddle the beam of light shining through your specimen slide.

4. Place a drop of immersion oil directly on the specimen slide on the beam of light. See Figure 7 below for the correct positioning the objective lenses and applying the oil.

![Fig. 7. Positioning the objective lens to apply immersion oil](image)

5. Again without lowering the stage or moving the focusing knobs, rotate the nosepiece until the **oil immersion lens (white ring, 100X)** touches the oil. You have just replaced the air between the slide and the lens with oil, thus reducing the refraction or bending of light.

You should now be able to focus the image with *minor* adjustments in the *fine* adjustment knob.
NOTE: Pay close attention so that you don't get oil on any of the other objective lenses, especially the 40X lens, or the stage. Wipe up any oil right away using only lens paper. If oil gets on the other objective lenses, the instructor may need to use a special cleaner before the lower power lenses can be used again.

![Image of human blood cells]

Fig. 8. Human Blood

6. The majority of cells in view are red blood cells. They are light pink and lack nuclei. The cells are biconcave and have faintly pink centers and darker stained edges. You will see a few larger, dark purple stained cells, the white blood cells. Notice that these cells have nuclei. The small purple cell fragments that are visible are called platelets and are involved in blood clotting. Draw a red blood cell, white blood cell and platelets as seen with the oil immersion objective.

7. When you have finished observing the slide, rotate the lens out of the oil and towards the 4X objective. Never move the nosepiece so that the high power objective (40X) goes into the oil.

Bacterial Specimens – If you take Microbiology (BIO 6), you will be looking at bacterial cells that are too small to focus on easily at low power. This requires a slightly different technique to get a good image at 1000X.

Obtain a bacterial specimen slide.

1. Before placing it on the microscope stage, hold the specimen slide against a piece of white paper and look for a colored haze or circle on the slide. When the slide was prepared, the bacterial cells were suspended in a drop of liquid, dried onto the slide, and then stained. Use the edge of this drop by positioning it in the beam of light coming from the condenser lens when you place your specimen on the microscope stage.
2. As usual, you will be starting with the stage at the highest position and the lowest power objective lens. The following procedure will help you to distinguish the bacterial cells from dust or other artifacts of the microscope:

Put your left hand on the focus knob and your right hand on the stage adjustment knob. While looking through the ocular lenses, slowly lower the stage with the coarse focusing knob in your left hand and simultaneously wiggle the slide adjustment knob back and forth with your right hand. Dust will be stationary, but your specimen will be moving as you move the slide adjustment knob. If an image appears and it is not moving, continue to focus with your left hand. If the image moves, you have successfully focused on the bacteria!. Use the fine focus to sharpen the image. Increase the magnification with the middle, high and oil immersion lenses as before. Once under oil, fine focus your image by looking through the ocular lenses and slowly rotating the fine focus knob by towards yourself. If the image does not sharpen within five or six half turns, change the direction of focusing. Count the turns until you are back to your original position, then another six or so half turns or until you can focus on your image. If you cannot focus within those six or so turns, ask for help.

![Fig. 9. Typical bacteria at 1000X](image)

3. **To Clean the Oil Immersion Lens**: ONLY lens paper should be used on any of the objective lenses to prevent scratches. Gently wipe a flat piece of lens paper ONCE across the oily surface of the lens. Turn the paper to a clean area and wipe again. Continue to turn the lens paper to a clean area with each wipe. Use a new sheet if the lens paper becomes too oily. Do not wad up the lens paper and scrub the lens. This may scratch the lens.
Remember when using the oil immersion lens: a. use only the fine adjustment knob  b. use only
the oil immersion objective with oil, do not use it dry  c. always keep the other objective lenses
free of oil  d. **blot the oil immersion lens with lens paper only when you are finished** e. wipe the
oil off the specimen slide with a Kimwipe

**STOP. Did you thoroughly clean the oil of the objective lens AND the slide?**

**G. Mitosis: The Cell Cycle**

Most cells go through a continual cycle of growth and replication called the cell cycle. The cell
cycle consists of four phases:

- a. **G1** or the initial growth phase
- b. **S** phase, during which the DNA is replicated
- c. **G2**, the second growth phase and
- d. **M** phase or mitosis, during which the cell divides its organelles, cytosol and
  replicated DNA among two identical cells.

Mitosis proceeds in four general stages:

1. **Prophase.** *(Pro means before)* During prophase, the nuclear membrane starts to
degenerate, and the DNA condenses so individual chromosomes are visible. Human cells have
23 pairs of homologous chromosomes: one set from the mother and one set from the father.
After the DNA is replicated, each homologous chromosome, exists in a set called sister
chromatids. Also during this stage, we see a structure called the mitotic spindle organizing
around the centrioles, which begin migrating to the opposite poles of the cell.

2. **Metaphase.** *(Meta means middle)* In metaphase we see the chromosomes line up along
the central portion of the cell. Spindle fibers branch from each side of the mitotic spindle and
attach to a structure called the centromere that joins each pair of sister chromatids.

3. **Anaphase.** *(Ana means away)* During anaphase we see the spindle fibers shorten, which
pulls the sister chromatids towards the opposite poles of the cell. In addition, a process called
cytokinesis begins, during which the cytoplasm is divided up among the two forming cells.

4. **Telophase.** *(Telo means end)* In the final phase of mitosis, telophase, a divot forms
between the two cells called the cleavage furrow. As the cleavage furrow progressively narrows,
the cell is pinched into two identical daughter cells. The nuclear membranes also begin to
reassemble, the mitotic spindle becomes less visible, and cytokinesis is completed.
Obtain a slide from the tray of Mitosis slides. You will observe the animal cell cycle in prepared slides of whitefish blastula.

a. Start out at low power. You should be able to see individual cells. Switch to medium and then high power so as to sketch and describe each of the stages of mitosis. For each stage indicate the magnification and describe what is happening within the cell (in detail).

H. Mitosis in Animals: A Study of Starfish Embryos  An egg that has been fertilized by a sperm is called a **zygote**. This zygote is now ready for repeated mitotic divisions that cleave the egg from a single cell into two cells, which cleave into four, then eight, then sixteen cells, and beyond, creating multicellular life. We’ll look at that process with starfish, observing the basic early stages that are shared by all animals, from earthworms to elephants, from whirligig beetles to whitefish (you just saw their blastula stage).

As cell division in an animal proceeds, the zygote transforms from being a simple sphere into a developing embryo with an obvious digestive tract. Below are listed the five general stages of animal embryonic development. You will be identifying and drawing these from slides you observe today.
1. **Unfertilized egg**- It’s a sphere with an obvious **nucleus** that takes up perhaps a fifth of its total volume. (The dark spot within is the **nucleolus**; it helps the nucleus make protein).

2. **Zygote**- The now-fertilized egg creates around itself a loosely fitting fertilization membrane that blocks additional sperm. The egg is still undivided, but now the diploid cell has lost obvious internal structures seen in an unfertilized egg.

3. **Morula**- Repeated mitotic cleavage produces this cluster of 16-32 cells that resembles a blackberry (*Mora* is Spanish for blackberry).

4. **Blastula**- As cells continue dividing, the embryo becomes a hollow ball of cells. The process is called **blastulation**. The cavity inside the cell is called a **blastocoel** (*blasto* relates to germination; *coel* means cavity).

5. **Gastrula**- This amazing stage is where the gastric tract forms, through **gastrulation**. It is created when cells at one end of the blastula start replicating while at the same time moving inward, creating an opening or **blastopore**. Replicating cells move deeper inside, creating an inward tube. As the tube extends deeper, the gastrula itself elongates, as you can see in the late gastrula stage shown below. The outer “skin” is called **ectoderm** and the cells migrating inside create a “tube-within-a-tube”. This “inner skin”, or **endoderm**, forms the digestive tract (This second cavity is called the **archenteron**).
I. Mitosis in Plants: Making Your Own Onion Tip Root Slide

*(Must use goggles and gloves for this section)*

Now you will observe the mitosis stage in a plant instead of animal cell. Actively growing onion root tips are required for this activity.

**Procedure**

1. Cut one root from an actively growing plant using a razor blade. *Caution:* The razor blade is extremely sharp.
2. Trim the tip of the root to 1 cm; use only the tapered end of the root tip.
3. Use forceps to place the root tip (use only the 1-cm tips) into a microfuge tube provided.
4. Using a clean, graduated pipet, add 2–3 drops of 1 M hydrochloric acid to cover the root tip in the tube. *Note:* Hydrochloric acid is corrosive to skin and eyes.
5. Label your microfuge tube. Place the tube in a rack into a 60 degree water bath. Leave for 10 minutes.
6. Use the pipet to remove the HCl and dispose of it in a glass waste beaker.
7. Add 2–3 drops of deionized water to the root tips.
8. Remove the water with a pipet and add 2–3 drops of stain onto the root tip. This may be acetocarmine or methylene blue or feulgin. *Note:* Methylene blue stain is a permanent stain.
9. Allow the root tips to soak in the stain for 3 minutes.
10. Remove the stain with a pipet and dispose of it in the glass waste beaker.
11. Add 1 drop of deionized water to the root tip to rinse.
12. Place a coverslip on the root tissue. Using your finger, gently apply pressure on the cover slip to squash the root tissue. Apply an even downward pressure on the root tips and cover slip but not so hard as to break the cover slip. Do not twist or grind the cover slip.
13. Using low magnification on the microscope, focus on the root cells. Switch to medium power or high power as necessary to easily visualize the inside of the onion root cells.
14. Study all of the squashed tissue to locate cells. The nuclei will be well stained, allowing you to search for cells actively dividing. See if you can recognize the stages you saw previously on the whitefish mitosis prepared slides. *Note:* All stages of mitosis may not be present within your slide. Note the difference between slides prepared with 2, 5 and 10 day roots by visiting other scopes in the class.
Be prepared to show your instructor the stages of mitosis as you find them on the onion root tip.

**SCIENTIFIC DRAWING GUIDELINES**

Often you will be asked to draw what you see either under the dissecting scope of the compound microscope. Here are some guidelines as to what is expected from a scientific drawing. There is no need to be a brilliant artist. With attention to detail and patience, these guidelines will help you create a very good scientific drawing. Your subject will be a flower. Use any flower that is provided in class, or one that you collected or brought with you.

1. **Drawing materials:**
   - A sharp HB pencil. Do not use pen or colored pencils.
   - Good quality unlined paper.

2. **Positioning:**
   - Center the diagram on the page. This leaves space for titles, labels and annotations to be added.
   - Do not put the diagram in a corner of a page.

3. **Size:**
   - The diagram should be large enough to represent all the details in the specimen without crowding them.
   - The minimum size is about 1/3 of a page. Rarely is a diagram too large.

4. **Accuracy:**
Your diagram should:
   - Be accurate and demonstrate your understanding of what you have observed
   - Be representative of what you see. You do not have to draw the entire field of view but rather you should draw enough to show the detail of the specimen and the relationships of the various parts of the specimen to each other.
   - If asked to draw ONE cell, make it a representative cell of those viewed, don’t pick out an unusual cell.
   - Sometimes it is necessary to draw the entire field of view but usually your instructor would indicate this.
• You will often be asked to draw a sufficient number to show their arrangement. You may have to use your judgement here, but usually 4-10 cells is sufficient.
• Show accurate proportions of the specimen

5. **Technique:**
   • Lines should be simple, narrow, sharp and firm.
   • When lines indicate an outline then the ends of the line should meet.
   • Represent depth only when necessary by stippling (dots as seen to the right). Do not use shading. Use the dots instead.

6. **Labels:**
   • Leave plenty of margin for labels.
   • Label lines must be ruled and they should not have arrow heads on the end.
   • Label lines should sit ON the structure they are indicating, not beside it.
   • The labels should appear at the end of the label line, they should not sit on the line.
   • As far as possible labels should be parallel and horizontal and vertical.
   • Names of structures must he horizontal.

7. **Title, Size and Scale conventions**
Microscope drawings should include the following (unless told otherwise):
   • A title, which should identify the material (organism, tissues or cell/s) and if appropriate the **stain** that was used in preparation. Singular/plural forms of words matter here since the title should indicate precisely what the viewer is seeing. For example, if one cell is drawn and the title says cells, then this will confuse the viewer who will be trying to make out where the cells are.
   • The magnification under which it was observed.
   • A scale to indicate the size of the object.
   • In the case of living materials, a brief description of any movement that was observed
   • Other annotations neatly made beside the drawing that communicate observations made.
J. Further Practice with Prepared Cells. Your instructor will guide you as to which of the following specimens you should observe.

Cork:
Cork is derived from the outer bark of *Quercus suber*, the CORK OAK tree. Obtain some cork and make a very, very thin flake of the cork about the size of a sun flower seed. Place a drop of water on the the cork and cover with a cover slip. Note the cubical cells, devoid of any living contents. You will most likely see these on the edge of your flake, where the cut is thinnest. Robert Hooke first coined the term "cell" from observing cork tissue! All that remains is the cell wall, which is heavily impregnated with **suberin**, a very water resistant compound. **Draw a section with approximately 10 cells.**

![Cork Image]

Ice Plant:
The epidermis of a plant functions to control the loss of water. It accomplishes this primarily through the excretion of a waxy layer called the cuticle. Like a sheet of wax paper, the cuticle limits the passage of water. In doing this it also blocks the passage of gasses between the plant and its environment. To keep the tissues from suffocating, the epidermis must have openings. These openings are called stomata (singular = stoma).

Make a wet mount of an epidermal peel of the stem of an ice plant. First cut the ice plant in cross section and then with forceps (or fingers), peel the outermost layer (epidermis) off the stem of the plant. Place the thin membrane on your slide, wet it with one drop of water, and cover with a coverslip. Observe the stomates (= stomata), containing two guard cells which control the opening (stoma). **Draw the stomata in your workbook.**

![Ice Plant Image]
Potato Amyloplasts (Starch Grains):
Plants store energy in the form of starch. Starch grains are visible within potato cells if sections of potato are sliced very thinly and observed under the microscope.

Obtain a small piece of potato (*Solanum tuberosum*) and make a couple of very thin, round slides about the size of a dime. Place your cut section on a slide, and with a pipet, dispense one drop of IKI over your potato slice. It will turn black within seconds. Cover with a cover slip. Now use a pipet to dispense water under the coverslip, and it will dilute the black iodine coloration making the starch grains more visible. Start by focusing on low power and then move to medium and then to high power. Observe the globular starch grains, often superimposed upon each other. Refer to drawing, and draw what you see on your own slide. It will never be exactly like the picture.

K. The Dissecting Microscope

A dissecting microscope has a longer working distance, and lower magnification than a compound microscope. This can allow you to get in and manipulate a specimen, and can be good for dissections. The trade off of course is lower magnification, but sometimes it is the lower magnification you want to be able to see structures in context. Our dissecting scopes have two ocular (eye) pieces that each use a separate path of light - allowing for true binocular vision which translates to good depth of field. There is a zoom knob (increases or decreases magnification) as well as a focus knob. Most will also have two light sources - one from above and one from below. Try both of these as each will allow for a different perspective into your specimen. Here, we will look at insect wings, using common lab tools (tweezers, pointer, razor blade etc.), but looking through the dissecting microscope. Time to be a surgeon - slow, careful manipulation of the specimen will yield the best results!
Insect Wing Patterns

Insect wings can be very varied and interesting! Compare the complex wings of the dragonfly to those of a small fly in the pictures below. Entomologist do much of their insect identification using wing patterns.

Using forceps, obtain an insect (dead one!) that your instructor has for you, or that you have collected from home or outside and brought in to class. If you get a beetle, it is the inner wings that you will be looking at, not the hard, outer wings.

a. Orient the insect on the stage of your microscope so that the insect is in a position that allows you to see its wings. (Easier said than done sometimes!)

b. Make a drawing of the overall shape of the wing, plus, all the veins that make up that wing. (Best to not pick a dragonfly!)

c. Notice that using the lower light on your dissecting scope will shine light through the wing, making it easier to see.

d. Can you find three of the structures labelled in the diagram below? Label those on your drawing.
e. Some vocabulary: **Cell**- enclosed box-like area of wing. **Basal cells** are boxes by the base of the wing and anterior (to the front). **Anal Cell**- is the box by the base of the wing but posterior (to the back). **Vein**- the lines within the wing. **Cross vein**- a perpendicular line on the wing. **Costa**- the main leading anterior vein. The **Sub Costa** is parallel to the costa. **Alula**- the bulge near the base of the wing.

f. Congratulations, you are an insect wing expert!

**Insect Legs and Body**

Look at your insect’s legs. Answer the questions in the workbook relating to the legs, thorax and abdomen.

Now, you have finished!

THE END