

# Human Anatomy & Physiology I

## Lab 4 Microscopy I – Basics of Microscopy

### Learning Outcomes

- Set up a microscope to view slides properly, efficiently, and without damaging the microscope or slide.  
**Assessment:** Exercises 4.1, 4.3
- Apply concepts of resolving power, resolution, lens magnifying factor, and total magnification to images viewed under a microscope.  
**Assessments:** Exercise 4.2
- Understand how a microscope changes and distorts an image.  
**Assessment:** Exercise 4.4
- Convert a visual image from a slide into an accurate but simplified line drawing.  
**Assessments:** Exercise 4.5

### The parts of a compound microscope and how to handle them correctly

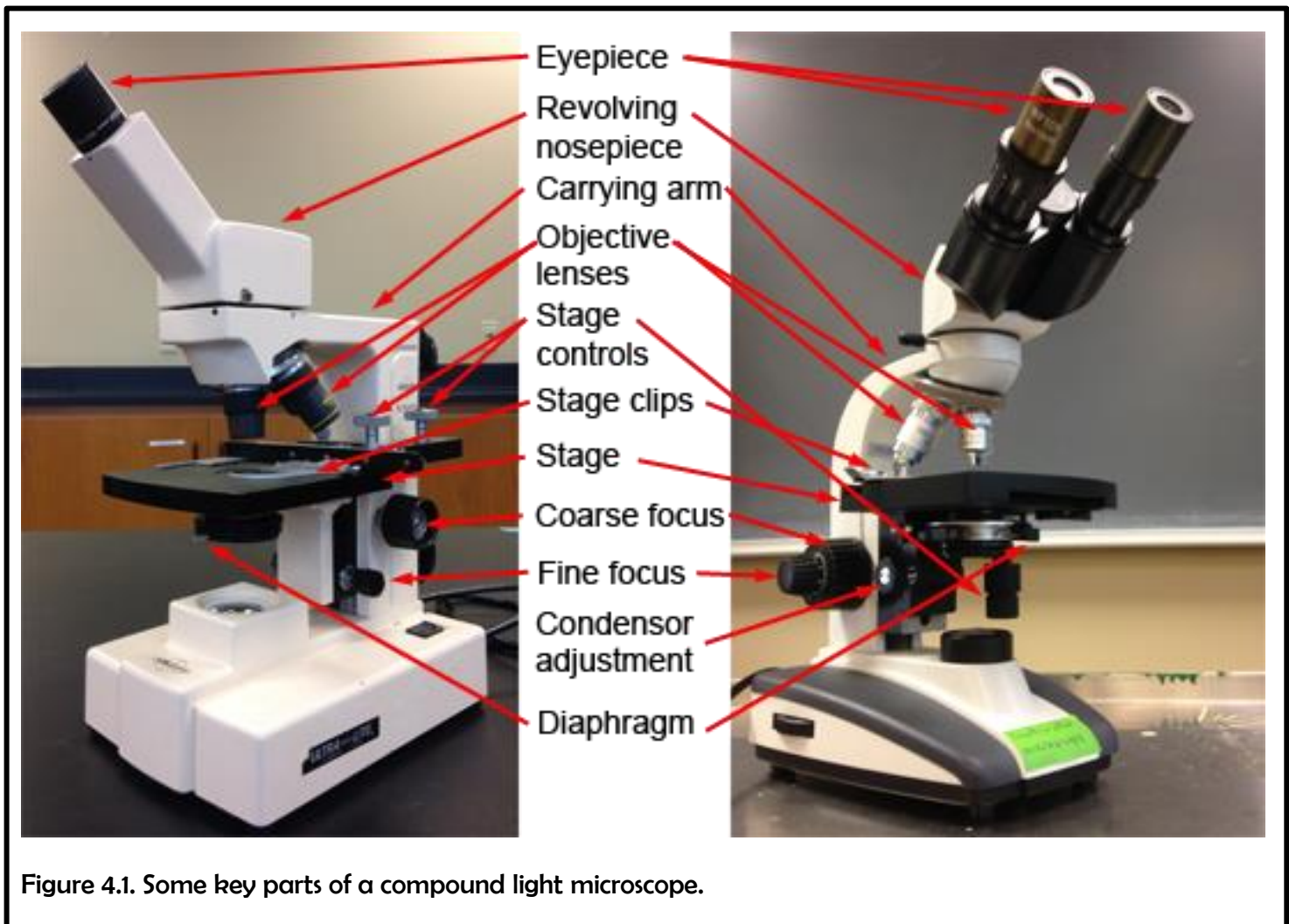
#### Information

Many important anatomical features, especially those that function at the tissue or cellular levels, are too small to be seen by the unaided eye. The compound microscope is a valuable tool for magnifying small sections of biological material so that otherwise inaccessible details can be resolved.

There are many different types of microscopes. We shall only learn about the compound light microscope. It uses visible light to visualize the specimen, but passes that light through two separate lens to magnify the image. The compound microscopes we will use in this course are sturdy instruments but they still have a lot of moving parts. They can be damaged and broken through misuse and mishandling. A large part of learning how to use the microscopes properly involves learning how to avoid damaging it. To do that, you first have to know which parts are which. Figure 4.1 identifies the key parts of the microscope that you need to be familiar with.

In Figure 4.1, there are two compound microscopes shown. The one on the left is monocular and the one on the right is binocular. Many of the parts of the two microscopes are in slightly different locations. Get used to this. Different brands and different models of microscopes position the key parts differently.

When you first sit in front of a microscope, you should always take a second to find the key parts, especially the focus knobs, the condenser adjustment knob (if present), and the stage control knobs. When viewing a specimen, your eyes will be at the eyepieces, and if you grab the wrong knob by accident, you can lose your image at best, and damage the microscope at worst. Don't assume you remember where the key knobs are. You may have a different microscope than last time.



### The eyepiece

This is where your eyes will be. If the microscope is binocular, use both eyepieces. With binocular microscopes, you almost always can adjust the width of the eyepieces to ensure they fit the spacing of your eyes. The eyepiece contains the eyepiece lens, one of the two lenses doing the actual magnifying in a compound microscope.

### The carrying arm

When moving a microscope, even if it is just a few inches, always pick it up by the carrying arm. Do NOT drag the microscope: pick it up. The microscope will have rubber feet that prevent it from sliding, so if you try to drag it, it will shake and vibrate and possibly damage parts. Never pick up the microscope by any part other than the carrying arm. The other parts are generally much more fragile and prone to breaking if you try.

### The objective lenses

Most compound light microscopes will contain three to four objective lenses that can be rotated over the slide. Sometimes these lenses are just called objectives. When a particular objective has been fully rotated into position, you will hear or feel a click as that objective locks into place. The objective lens is the second of the two lenses doing the actual magnifying in a compound microscope, so if it is not snapped into proper position, you won't see the proper image. Each

objective lens can usually be unscrewed from its position in the rotating turret that houses it. Be careful you are rotating the turret, not unscrewing an objective. Do NOT unscrew the objectives from the turret. Each objective lens has a different magnifying power, so the image on your slide will be magnified to lesser or greater extents, depending on which objective lens you have chosen. Each objective's magnification power will be written somewhere on the side of the objective, although sometimes it is hard to see the number. The magnification of an objective lens will always be a whole number. There will be other things written on the side of an objective, but the one that is a whole number greater than 1 will be the magnification. You can ignore everything else written there.

## The stage

The stage is the platform that the slide will be clipped on to.

## Stage clips

The slide will be held in place on the stage with stage clips. Most of the time, these will clip against the sides of the slide. They do not sit above or below the slide. They are spring-loaded to hold the slide edges and lock the slide in place so that the stage controls can move the position of the slide smoothly. If the slide is not clipped in place, you won't be able to reposition the slide to find microscopic features of interest.

## Stage Controls

These allow you to move your slide while you are viewing it, but only if the slide is properly clipped in with the stage clips. Always find where these are on your microscope before you start viewing your slide. They seem to never be in the same place in two different microscopes and if you just blindly grope for them while viewing your slide, you will likely do something unfortunate to your view or to the entire microscope. There are always two dials. One moves the slide left and right. The other moves the slide up and down. Sometimes they are on top of each other, as in the binocular microscope in Figure 4.1. Sometimes they are two separate dials, as in the monocular microscope in Figure 4.1. Sometimes they are above the stage, as in the monocular microscope in Figure 4.1. Sometimes they are below the stage, as in the binocular scope in Figure 4.1. Spend a few seconds to find them every time before you sit down at a microscope.

## Coarse focus

This is always the larger of the two focus knobs. You should usually only need to use the coarse focus knob once for each new slide. Use it with the lowest power objective to get the specimen approximately in focus. After that, only use the fine focus knob, even after you change to a higher-power objective. Sometimes the coarse focus knob is with the fine focus knob, as on the binocular microscope in Figure 4.1. Sometimes it is separate from the fine focus knob, as on the monocular microscope in Figure 4.1.

## Fine focus

This is always the smaller of the two focus knobs. This is the focus knob you will use over and over again in viewing slides. Don't change the coarse focus after using it for the first time, only change the fine focus.

## Condenser adjustment

Not all microscopes have a condenser adjustment knob. If there are only two knobs, as on the monocular microscope in Figure 4.1, those two are the coarse focus and the fine focus and you only

have to keep those two separate. But if there is a third knob, it is the condenser adjustment knob. As a general rule, do NOT touch or adjust this knob. It controls how far the light condenser is from the slide, which should be properly adjusted before you use the microscope. If you move it, you will have it in the wrong position. If your scope has the knob, find out where it is and avoid it.

### Diaphragm

This is directly under the hole in the stage where light passes through to the slide. It is controlled by a level which opens and closes an iris to let more or less light through the slide. In some specimens there is not much contrast between the colours and shades of the different components being magnified. Changing how bright the view is by adjusting the diaphragm can allow you to better see some of the details you are trying to magnify.

### Lab exercises 4.1

There is a compound microscope for each student. Carry out the activities listed below and fill in the blanks as you do so.

1. Pick up your microscope and physically move it to a new location. Bring it close enough that you can look into it comfortably from where you are sitting. Arrange it so that the stage is facing you and the eyepiece is rotated towards you. What part of the microscope did you grab in order to pick it up and move it?  
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2. Where are the locations of the two stage adjustment knobs on your microscope?  
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3. Where is the location of the coarse focus knob? \_\_\_\_\_

4. Where is the location of the fine focus knob? \_\_\_\_\_

5. Is there a condenser adjustment knob? If so, where is it located? \_\_\_\_\_

6. Find the diaphragm lever. Looking in the hole in the center of the stage, what happens when you slide the diaphragm to the right?  
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7. Still looking down at the hole in the center of the stage, what happens when you slide the diaphragm to the left?  
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## Magnification and resolution

### Information

The reason for using a microscope is to magnify features to the point where new details can be resolved.

**Magnification** is the factor by which an image appears to be enlarged. It will be a whole number greater than 1 and is usually followed by an “x”, as in 10x magnification.

When you look through microscope eyepieces, you are seeing a **virtual image** because in reality, what you are looking at is not as large as it appears through the eyepieces, and because there can be some distortion of the image.

**Resolution** is the shortest distance between two points that can still be visually distinguished as separate. The resolution of a typical unaided human eye is about 200  $\mu\text{m}$ . Using a microscope decreases the resolution to distances as short as 0.2  $\mu\text{m}$ . Resolution is a property of the eye.

**Resolving power** is the ability of a lens to show two adjacent objects as discrete. Resolving power is a property of a lens.

Each lens in a microscope has a **numerical aperture**, or NA, value. This has to do with the angles of light that enter and exit a lens. Its applications are beyond the scope of this lab, but numerical aperture does influence the resolution possible with a particular lens, and so the NA value for the lens is usually printed on each objective. It will be a number less than 1.0, and you can ignore it for our purposes.

Each lens in a microscope also has a **magnifying factor**. This is the degree to which that lens magnifies an image. It will be a number larger than 1.0. For instance a 10x objective magnifies the image ten-fold. The magnifying factor for each objective always printed on it, and the magnifying factor for each eyepiece is usually printed on it. (If the eyepiece is missing a printed magnifying factor, you can usually assume it is 10x.)

The **total magnification** for any image viewed under a compound microscope is calculated by using the formula:

$$\text{Total Magnification} = \text{eyepiece magnifying factor} * \text{objective magnifying factor.}$$

So, each time you switch objectives, you change the total magnification. Total magnification does not have units, but is usually indicated by an “x”, as in “total magnification = 100x.”

## Lab exercises 4.2

1. Write down the magnification factor for the eyepiece lenses on the microscope in front of you.

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2. Using the microscope in front of you, write out all the words and numbers written on each objective on your microscope. There are probably three objectives, but some microscopes might have four. Start with the smallest objective and move through them in order of increasing size.

Objective one \_\_\_\_\_

Objective two \_\_\_\_\_

Objective three \_\_\_\_\_

Objective four \_\_\_\_\_

3. In the above list, for each objective, circle just the magnification factor for that objective. Remember, the magnifying factor is a whole number, and differs for each different objective.
4. Write down the total magnification (eyepiece magnifying factor \* objective magnifying factor) when using each objective on the microscope in front of you.

Objective one \_\_\_\_\_

Objective two \_\_\_\_\_

Objective three \_\_\_\_\_

Objective four \_\_\_\_\_

5. If you observed two features on a slide with your naked eye that were 0.5 mm, how far apart would they appear to be if you observed them with the microscope in front of you, using the second objective?

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## Setting up a microscope and slide properly

### Information

All the microscopes in the lab are **parfocal**. That means that if the slide is in focus under one objective, it will stay largely in focus if the objective is changed. In practical terms, this means you should usually only need to use the coarse focus knob once per slide. You get the slide in focus under the lowest-power objective (where focusing is easiest), then, from that point onward, only make minor adjustments with the fine focus knob even if you change objectives.

When you first get a new slide, you can usually determine the location of the specimen by looking at the slide while it is still in your hand. The specimen is usually a patch of colour somewhere near the center of the coverslip. After you clip your slide securely onto the stage with the stage clips, use the stage control knobs to move the patch of colour until it is directly over the hole in the center of the stage where the light comes through. Now when you look through the eyepieces using the lowest objective (always start with the lowest objective) you should be able to find the specimen and get it quickly in focus.

Occasionally the eyepiece or objective lenses will have specks of dirt or dust on them, making it difficult to focus on the specimen. To clean lenses, always use lens paper supplied by the lab instructor or a pure cotton swab. Do not use any other type of cloth or paper as they might scratch the lens. To remove dirt with lens paper, first roll up the lens paper and try to dry brush away the dirt in a spiraling motion that circles from the center of the lens out. If that doesn't work, moisten the lens paper or pure cotton swab with water (do not apply the water to the lens directly) and clean in a spiraling motion from the center of the lens out.

On the next page is a checklist for initially setting up a microscope. Every time you get a new slide, you should use this checklist.

### Lab exercises 4.3

Get a slide with the letter “e” on it. Then following the checklist below, checking off each item as you complete it. For this slide, the letter “e” is the specimen we are interested in.

Checklist for setting up a microscope and beginning with a new slide.

1. Plug in microscope & turn on light source.
2. Pick up microscope by carrying arm, position it so it is accessible to your seat, with open side of the stage facing you.
3. Rotate the objectives so that the lowest power objective (smallest in size) clicks into place.
4. Look at slide with your naked eye and find the location of the specimen.
5. Clip slide into place with stage clips. The cover slip on the slide must be face up. Find the stage controls and make sure that, when they are turned, the slide moves smoothly left & right or up & down, depending on knob.
6. Use stage controls to move slide so that light source is shining directly on to the specimen to be magnified.
7. Find the coarse and fine focus knobs. Watching the stage and objective, use the coarse focus knob to bring the low power objective as close to the slide as it will go.
8. Put your eye to the eyepiece (or eyepieces, if the microscope is binocular) and rotate the coarse focus knob in the lowering direction until some aspect of the specimen comes into focus.
9. Move your hand to the fine focus knob and get the specimen into perfect focus for your eyes. Do NOT touch the coarse focus knob again.
10. Use the stage control knobs to move your specimen to close to the exact center of your field of view.
11. Move to the next highest power objective (do not skip individual objectives) and use only the fine focus to get your image into perfect focus for your eyes.
12. If you need further magnification, move to the next highest power objective and use only the fine focus to get your image into perfect focus for your eyes.
13. Do not use the 100x objective (if you have one) in this course. It must be used with immersion oil and we won't have students doing that.



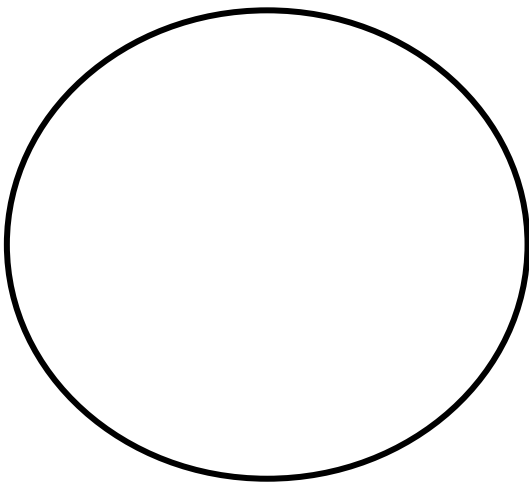
## How the virtual image differs from the real image

### Information

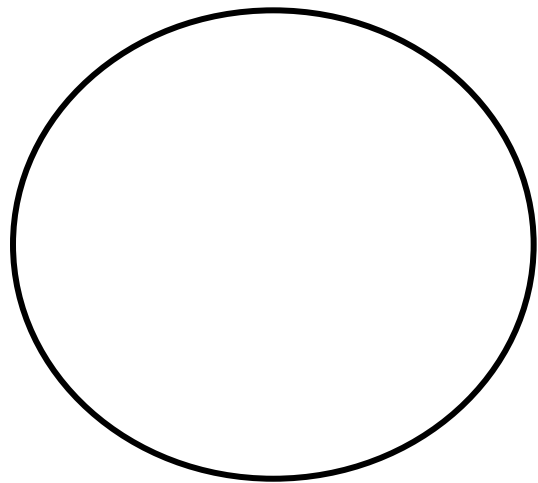
The virtual image you see when looking in your microscope is not quite the same as the real image you would see with your eye. For one thing, it is bigger. For another thing, the orientation of the image is different. The two lenses in a compound microscope reflect the original image two times, in two different planes, while magnifying it. So what you think of as the “top” of your image is really the bottom, and what you think of “right” is really left. Usually this is not an issue at the microscopic level, but it is important to understand how the microscope is rearranging your virtual image.

### Lab exercises 4.4

1. Get an “e” slide. If it is already under your microscope, rotate the lowest-power objective into place, use the coarse focus to lower the stage, and remove the slide.
2. Look at the unmagnified “e” on the slide by eye. Rotate the slide around in your hand so that the “e” is right side up. Now clip the slide onto the microscope stage with the stage clips so that the “e” is facing you right side up when you look at it with your unaided eye.
3. In the right-hand circle below, draw what the “e” looks like when you are looking at it right side up. Assume the circle below is the size of the entire coverslip. Draw the “e” you see unaided in the correct proportion to the coverslip. (The unmagnified “e” will take up a tiny portion of the coverslip area.)



Total magnification: \_\_\_\_\_



Total magnification: \_\_\_\_\_

4. Clip the slide into your microscope stage so that the “e” is still facing you right side up. Follow the checklist in Lab Exercise 4.3 to ensure you are setting up the microscope properly for use, but stay on the lowest power objective. Get the “e” into your field of view and in focus.
5. In the left-hand circle above, draw what the “e” looks like when viewing it through the microscope under the lowest-power objective. Under the circle, write the total magnification of the image.
6. When viewed under a microscope, how is a specimen rotate? \_\_\_\_\_

7. Look at the stage and slide directly (not through the eyepieces.) Move the stage control knob that causes the slide to move away from you on the stage, then move it back to its original position.

8. Now move the stage control knob the exact same way you just did, but view the “e” through the eyepiece. When the stage is moving away from you, what direction does the virtual image appear to be moving?

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9. Again, look at the stage and slide directly (not through the eyepiece.) This time, move the stage control knob that causes the slide to move to your right, then move it back to its original position.

10. Now move the stage control knob the exact same way you just did, but view the “e” through the eyepiece. When the stage is moving to your right, what direction does the virtual image appear to be moving?

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11. The **field of view** is the entire area you can see when looking through an eyepiece. Use the stage control knobs to move the virtual image of your “e” to one side of the field of view. Keep most of the “e” in the field of view, but move it to one side or the other.

12. Now switch to the next-power objective. (Do not skip.) To get to the next-power objective, and not the highest-power objective, which way did you have to rotate the objectives, clockwise or counter-clockwise?

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13. Using only the fine focus knob (you do NOT use the coarse focus knob on any objective other than the lowest objective), get the “e” in focus.

14. When moving to the next objective, which part of the field of view do you zoom in on?

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15. Move away from the eyepieces and look at the distance between the slide and the bottom of the objective. Rotate back to the lowest power objective. Now rotate to the next objective (do not rotate to the highest-power objective by accident). Now rotate to the third-highest objective. What happens to the distance between the slide and the bottom of the objective as you rotate to higher power objectives?

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16. With the third-highest power objective still in place, how space is there between the slide and the bottom of the objective?

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17. Notice there is a danger of smashing the objective lens into the slide if you were to use the coarse focus. Why are you instructed to only use the coarse focus with the lowest-power objective?

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## Making simple but accurate line drawings of magnified specimens

### Information

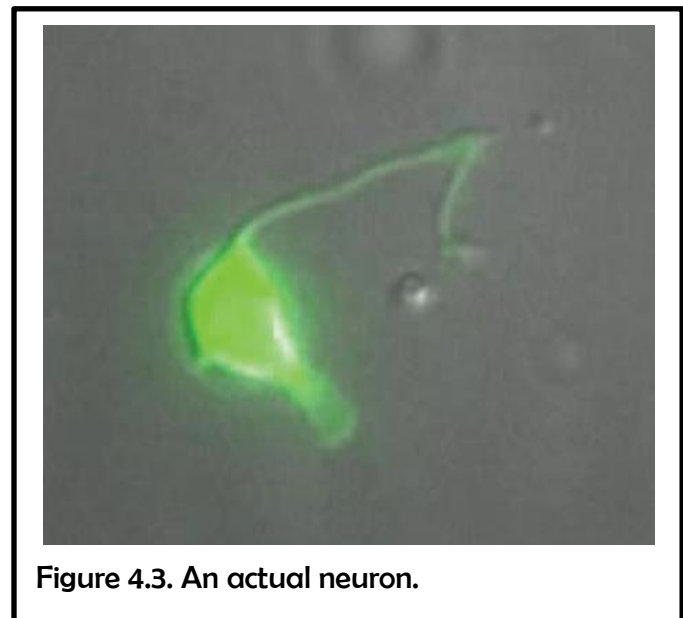
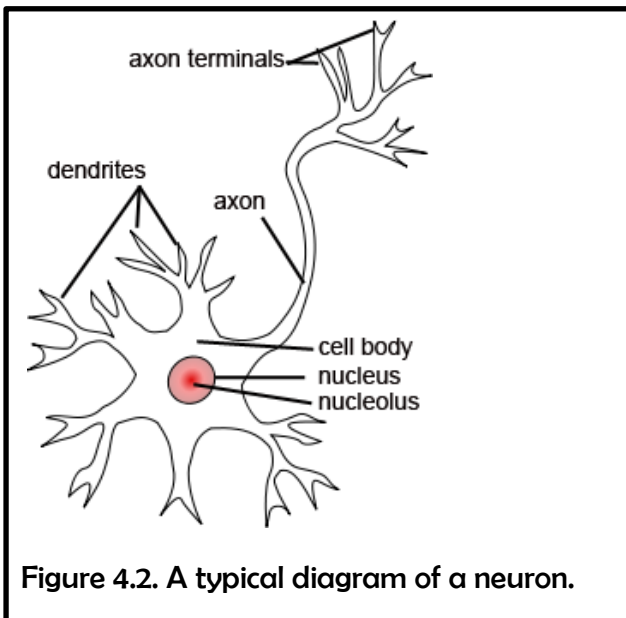
You do not have to be a great artist to make a diagram of the cells and structures you see under a microscope. You only have to be careful to draw something that is approximately the same size and shape as what you see. Follow the following guidelines:

1. Only draw what you actually see. Even if you expect to see something, if it is not there you should not draw it. Do not base your drawings on what the textbook or some other source tells you should be there. Do not draw things in the shapes that texts or other sources tell you to expect unless you actually see those shapes.
2. Keep things as simple as possible. Draw strong unbroken lines. Avoid shading or cross-hatching unless there is a very good reason to add them.
3. Feel free to simplify reality by leaving out unnecessary details. Draw what is of interest, but leave out background material, debris, or any other distracting items. Just be careful that if you are leaving something out that it isn't something that is an important part of what you are drawing.

You should always have a basic understanding of what you are looking for before looking in the microscope. Tissues and other microscopic specimens can be confusing and cluttered. If you know in general what you are looking for, and, sometimes more importantly, what you are **not** looking for, it will make it much easier to find what you want to draw and it will make it much easier to decide how to draw it.

Just remember, what you see under the microscope may look quite different from the perfect specimens that are usually found in the figures put into textbooks and websites. Use the idealized images to track down what you are looking for, but draw the specimen as it actually is, regardless of your expectations.

For instance, in most textbooks, neurons, the most common cell found in nervous tissue, are drawn to look like variations of this:

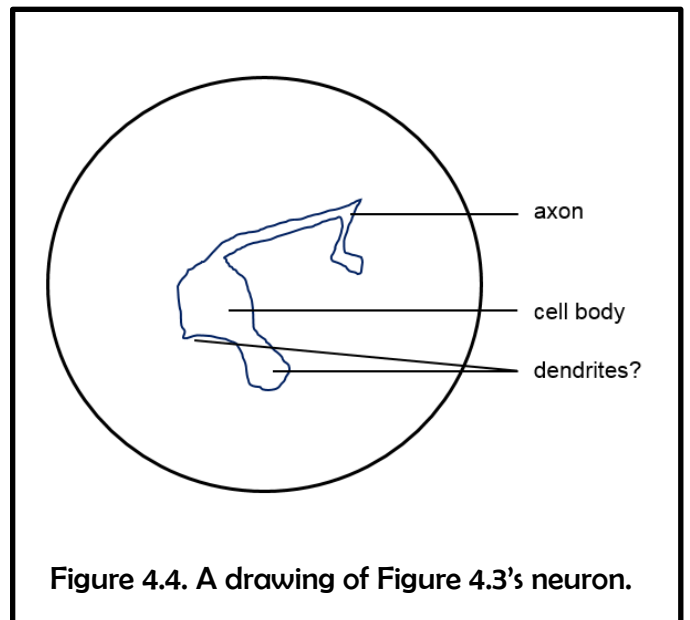
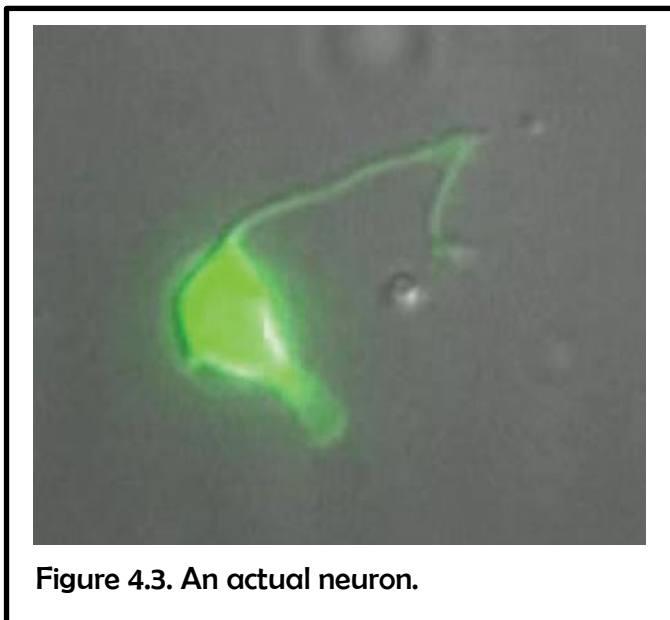


In the typical diagram of a neuron that appears in texts and on websites, there usually is a clear nucleus, and often a nucleolus visible, too. Sometimes organelles such as mitochondria are visible (there are none in Figure 4.2.) The dendrites are typically short and branched. There almost always is a single, easily-identifiable axon that is longer than all the dendrites and that branches as it ends.

Figure 4.3 shows an actual neuron as viewed through a microscope. If you view enough neurons through enough different types of microscopes, you can eventually create a composite diagram that incorporates features from many specimens to present a “typical” neuron, but it is unlikely that if you view a single neuron you will see everything in in Figure 4.2. In fact, often actual specimens look very little like their textbook counterparts. Draw what you see, not what you think you are supposed to see. Just make sure you are looking at what you are supposed to be finding (for instance, a neuron and not a piece of dirt or cell debris), and then draw it as it is, regardless of whether or not it

In the case of the neuron in Figure 4.3, there is no nucleus visible, there is maybe one large projection and one small projection you could call dendrites – but there aren’t many projections – and neither of the projections are branched. There is one long thin projection that is probably an axon, and it is not branched.

If you draw what you see, you end up with a drawing like the one in Figure 4.4. It does not look like a textbook neuron, but it is a reasonable representation of what is there in this case.



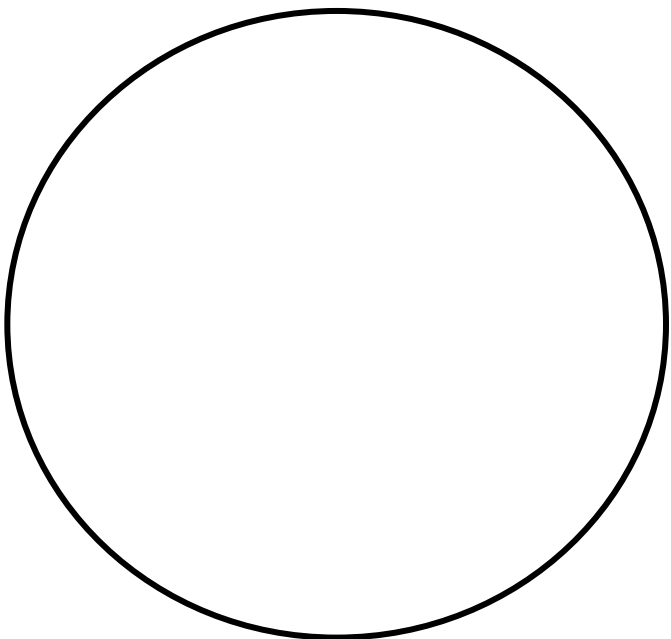
Most students feel they “cannot draw” and are reluctant to sketch what they are seeing under a microscope. You don’t let your lack of artistic skills stop you.

1. Draw an outline that approximates the item you want to draw. Don’t obsess about making it match perfectly. Approximate is ok.
2. Try to get the proportions approximately right. If something is half as big, or as third as big, as something else, make it that way in the drawing, too.

3. Do not draw everything you see. Improve on reality by only drawing the parts of the specimen you are interested in. You do not have to draw every bit of debris or dirt. Decide what the important parts of your specimen are and draw only those.
4. Do not use shading or cross-hatching unless there is a very good reason to do so. It will actually make it easier to understand your drawing if you stick to drawing just outlines. It will also be easier and quicker to draw.

### Lab exercises 4.5

1. Get a human blood smear slide. Rotate your lowest power objective into place on your microscope.
2. Follow the checklist in Lab Exercise 4.3 until you are viewing the blood smear under you 40x objective.
3. You will see mostly red blood cells. They will probably be pinkish and they will be the circles without nuclei. Occasionally, some will appear to have blank circles in their centers, but these are not nuclei. If you search around your slide using your stage controls, you will find the rare circular cells with nuclei. These are white blood cells. There will be less than one white blood cell for every 100 red blood cells. These white blood cells will probably be light blue or grey and have purple or dark blue nuclei. Their nuclei will not always be round.
4. Find a section of your slide with two or more white blood cells among all the red blood cells.
5. In the circle below, draw four or five representative red blood cells (do not draw all the red blood cells you see) and draw all the white blood cells in your field of view. Pay careful attention to drawing the white blood cell nuclei as accurately as possible.
6. Do not remove or change the position of your slide until the instructor has verified that your white blood cells are drawn accurately.



## Licenses and attributions.

Unless otherwise noted, all figures



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<https://commons.wikimedia.org/w/index.php?curid=18271454>

Figure 4.3 Source: modified from: <http://dx.doi.org/10.1371/journal.pone.0112102>

Isolation of specific neurons from *C. elegans* larvae for gene expression profiling.

Spencer WC , McWhirter R , Miller T , Strasbourger P , Thompson O , Hillier LW , Waterston RH , Miller DM 3<sup>rd</sup>. PloS one 2014, 9(11):e112102.

Figure 4.4 Source: created by R. Whitwam for this work.