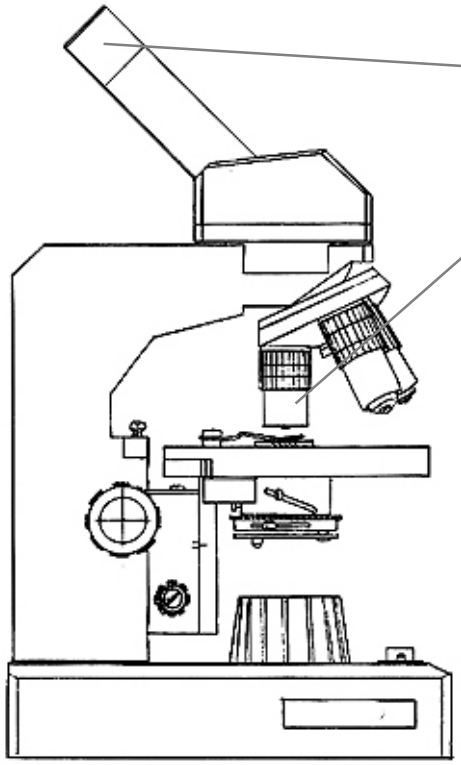
The Compound Microscope

a) Introduction

Ever since the invention of the first microscopes in the sixteen hundreds, they have become an invaluable tool in the study of living things. The goal of today's exercise is for you to become familiar with operating the microscope. The microscopy skills that you learn today will be useful not just with this course but in almost every biology course.

b) The Compound Microscope

The type of microscope that you will use is called the compound microscope. It is the most common type of microscope. It is called a compound microscope because the light passes through <u>two</u> different magnifying lenses, so the total magnification of the image is compounded.



<u>The ocular lens:</u> This lens is also called the eyepiece lens because it is the lens closest to your eyes. It magnifies the image 10X

<u>The objective lens:</u> This lens is called the objective lens because it is closest to the specimen (the object) that you are viewing. The microscope has four different objective lenses (4X, 10X, 20X, and 40X). Whichever lens you click into the vertical position is the one that you are using.

The total magnification of the image is obtained by compounding (multiplying) the magnification of the two lenses. For example, if you are using the 40X objective lens, the light passes through that lens (40X) and the eyepiece lens (10X), so the total magnification is 400X (40 * 10).

c) Getting started: Viewing a prepared slide

A microscope slide is the flat glass piece that holds the specimen. A prepared slide is one that has been prepared for you. Scientific supply companies sell prepared slides of hundreds of different animal and plant tissues.

Obtain a prepared slide of the small intestine (part of the digestive system). As you go through the steps of the viewing procedure, the microscope parts are listed in **bold** type. If you are not sure where any microscope part is, refer to the diagram on page 10.

1) Turn the objective lenses until the **4X objective lens** clicks into place, pointing straight down at the **stage** (the flat platform). Don't look into the eyepiece yet (that will come on step 8).

2) Turn the **course focus knob** (the largest dial on the side of the microscope) to make the distance between the **objective lens** and the stage as big as possible.

3) Put the intestine slide into the **slide carrier** (the metal clip on the stage). The slide carrier opens up and the glass slide clips into it. Do not wedge the slide under the slide carrier. This is not the correct way to hold the slide and it can damage the microscope.

5) Turn on the electrical power. Set the electrical power adjustment to make the light as bright as possible.

6) Use the two **mechanical stage knobs** (two knobs pointing downward, next to the stage) to adjust the slide position so that the specimen is in the "spotlight" coming up through the stage.

7) Turn the **course focus knob** to bring the objective lens as close to the slide as possible.

8) Now look into the eyepiece. If the light is uncomfortably bright, use the **condenser iris diaphragm lever** to make it dimmer. While looking into the eyepiece, turn the course focus knob slowly until the specimen comes into sharp focus.

Once you have the image in sharp focus, use the two mechanical stage knobs to scan around on the image. You are not required to know the intestine's tissues for this lab exercise, but just in case you are curious about them: The purple tissue that looks like tiny fingers (or island chains) sticking out is called the mucosa. It makes digestive enzymes and absorbs nutrients. Under the mucosa is a layer with white and light pink called the submucosa, and under the submucosa is a solid pink layer of muscle tissue that churns your food and pushes it through the intestine. This is called the muscularis externa.

c) How the microscope changes the image

1) Keep the intestine slide in the microscope, but while **not** looking into the eyepiece, turn a mechanical stage knob so that you see the slide move to the right. Now look through the eyepieces while continuing to move the slide to the right. Which way is the image moving? _____

2) While **not** looking into the eyepiece, turn a mechanical stage knob so that you see the slide move to the left. Now look through the eyepieces while continuing to move the slide to the left. Which way is the image moving? _____

3) While **not** looking into the eyepiece, turn a mechanical stage knob so that you see the slide move up. Now look through the eyepieces while continuing to move the slide up. Which way is the image moving? _____

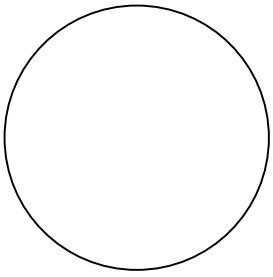
4) While **not** looking into the eyepiece, turn a mechanical stage knob so that you see the slide move down. Now look through the eyepieces while continuing to move the slide down. Which way is the image moving? _____

Summarize your findings by filling in the blanks in this sentence:

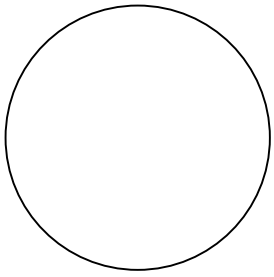
The microscope changes left to _____, right to _____, up to _____, and down to _____.

Now obtain a microscope slide with the letter "e" on it. **First, look at the slide with your eye (<u>not</u> with the microscope).** Confirm that the letter "e" is in the normal orientation (not upside down or backward).

Consider the sentence you wrote in the box above, then make a prediction about how the "e" will look under the microscope. Sketch what you think it will look like in the circle below on the left. Now follow steps 1 – 8 on page 2 to view the "e" under the microscope. Sketch what it actually looks like in the right circle. Did it match your prediction? **<u>Show your instructor your results before continuing.</u>**



Predicted "e"

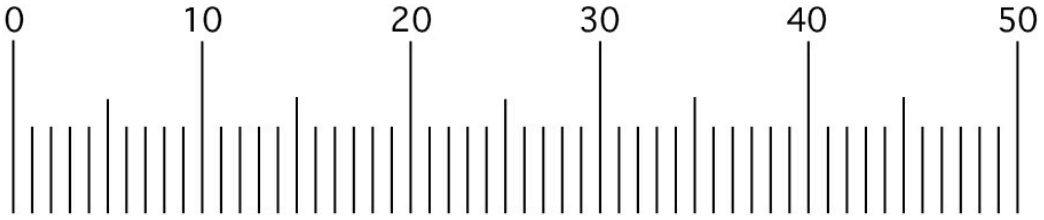


Actual "e" image

d) The field of view size

Notice that when you look through the microscope, the image is contained inside a round boarder. The diameter of this round viewing area is called the field of view. In this section, you will measure the diameter of the field of view (how many millimeters across it is).

Obtain a micrometer slide. Micrometer slides have a small ruler that can be seen under the microscope. Follow steps 1 – 8 on page 2 to view the ruler with the 4X objective lens. The ruler should appear like the one shown below:



Example: This rectangle
is 2.6 mm in length

The numbering on the ruler is slightly confusing: First of all, ignore the numbers on the ruler. Just focus your attention on the lines. The ruler has 50 lines total. Each line on the ruler is 0.1 mm from its neighboring lines. So to find an object's length in mm count its lines then divide by 10. As an example, the black rectangle shown above is 26 lines long, so its length is 2.6 mm (not 26 mm).

Now that you know how to use the ruler, your job is to find the diameter of your field of view.

1) View the ruler using the 4X objective lens.

2) Use the mechanical stage knobs to move the ruler's zero line to the far left edge of your field of view.

3) Now use the lines on the ruler to measure the distance from the left edge to the right edge of your field of view. This distance is the diameter of your field of view for the 4X lens. Write the diameter of the 4X lens' field of view and its total magnification in the table on page 10. Keep the ruler slide in place for the next activity.

e) The 10X (low power) objective lens

So far you have been viewing using only the 4X objective lens (sometimes called the "scanning" lens). In this activity, you will switch to the 10X lens (called the "low power" lens). Before switching to the 10X lens, first have the specimen (the ruler) in sharp focus and perfectly centered in the field of view.

Now click the 10X objective lens in place and view the ruler. Use the mechanical stage knobs to move the lines to the middle of your field of view. Now count the lines and divide by 10 to find the field of view diameter. Write the diameter of the field of view and the total magnification on page 10. Notice that the 10X field of view is *smaller* than the 4X field of view. This seems confusing at first since the 10X is a more powerful lens. But consider it this way: Since the 10X has a higher magnification then the 4X, it zooms in on a *smalle*r part of the specimen, which means the 10X is showing you a *smaller* portion of the slide, so it must have a smaller field of view.

Lastly, switch back and forth between the 4X and the 10X. Try to decide which lens gives a brighter image and which gives a dimmer image. Record your finding on page 10. **Show your instructor your results before continuing.**

f) The 20X (medium power) objective lens

Before switching to the 20X lens, first you must have the specimen in sharp focus and perfectly centered in the 10X field of view.

Now switch to the 20X lens. Use the same measuring method that you used before to measure the diameter of the field of view. Write the diameter of the 20X lens' field of view and the total magnification in the table on page 10. Lastly, switch back and forth between the 10X and the 20X. Try to decide which lens gives a brighter image and which gives a dimmer image. Record your finding on page 10. **Show your instructor your results before continuing.**

g) The 40X (high power) objective lens

In this activity, you will switch to the "high power" 40X lens. Students often have a hard time viewing specimens with the high power lens, but by following the two instructions below, you can avoid many of the difficulties.

1) Before switching to the 40X lens, first you must have the specimen in sharp focus and perfectly centered in the 20X field of view. (If the specimen is not centered and in focus, it is almost impossible to see it when you switch to 40X).

2) After you have focused on 20X, DO NOT change the focus knobs when you switch to 40X. Simply turn the lenses until the 40X clicks into place. After you have switched to 40X, you may need to fine-tune the focus slightly. Use only the **fine focus knob** (the small knob inside the course focus knob) to fine-tune the

focus. The course focus knob changes the focus too rapidly to be useful at 40X. Also, using the course focus can cause the high power lens to ram into the slide, damaging the lens.

Following the two instructions above, view the ruler under the 40X lens. Use the same measuring method that you used before to measure the diameter of the field of view. Write the diameter of the field of view and the total magnification in the table on page 10. Lastly, switch back and forth between the 20X and the 40X. Try to decide which lens gives a brighter image and which gives a dimmer image. Record your finding on page 10. **Show your instructor your results before continuing.**

h) Making a wet mount slide, part I

In this activity you will make and view a slide containing your own cheek cells (from the inside of your mouth) in a drop of water. Any slide that you prepare yourself by putting a specimen in a drop of water is called a wet mount slide. Since this wet mount slide will contain human tissue, for safety reasons only you should handle your slide.

1) Obtain an empty glass slide and a cover slip (a small thin plastic square about the size of a postage stamp).

2) Mix a drop of de-ionized water and a drop of methylene blue stain together on the slide. Stains are special colored liquids that make parts of the specimen stand out.

3) Obtain a toothpick. Use one end to scrape the inside of your cheek. The harder you press it against your cheek, the more cells you will collect, but of course don't injure yourself.

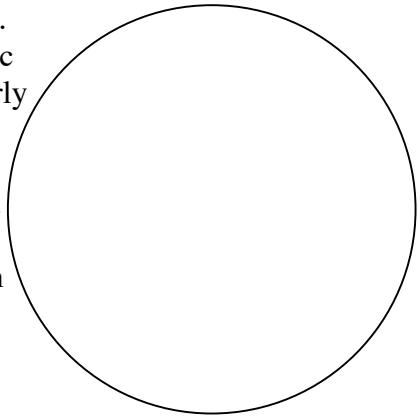
4) Swirl the end of the toothpick in the drop on your slide. The cheek cells will move into the liquid and become stained. Discard your toothpick into the trash.

5) Place the cover slip on top of the drop. In other words, your cells should be sandwiched between the slide and the cover slip. The cover slip holds the cells flat and protects the lens from the liquid on the slide.

6) View the cheek cells at 10X using the steps 1 – 8 on page 2. Often, many cheek cells are bunched together in large clusters. Scan around on the slide until you find an individual isolated cheek cell.

7) Bring the isolated cell into sharp focus and exactly center it in your field of view. Now switch to the 40X lens (we will skip the 20X). You may have to focus using the fine focus knob.

8) The dark blue spot in the middle of the cell is the nucleus. It is dark because methylene blue stains DNA. If you look carefully, you may faintly see other organelles besides the nucleus. (It would take other stains and a more powerful microscope to precisely identify these organelles. Biologists sometimes use the phrase "cytoplasmic granules" to mean organelles that cannot be clearly identified).



9) Sketch the cheek cell in the circle on the right. Label the nucleus and the cytoplasmic granules. Next to your sketch, write the total magnification that you are using while sketching.

10) For now, set the slide aside. You will view it again in section J below.

i) Making a wet mount slide, part II

In this activity you will make another wet mount slide, but time you will be viewing some microscopic organisms that live in water. The procedure is similar to what you did before, except that you will mix the water with a substance called ProtoSlo, which makes the water more viscous to slow down the organisms for better viewing.

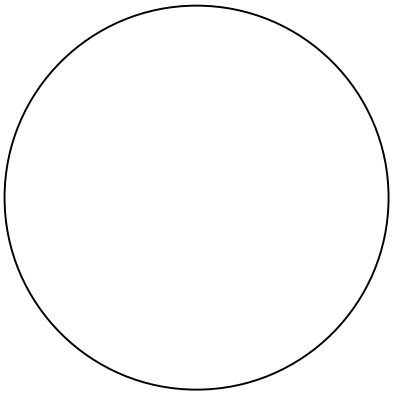
1) Obtain a slide and a cover slip.
2) Find a jar full of pond water. Using a pipette, suck up some of the green scum at the bottom of the jar. Transfer a drop of the pond scum to the slide. Also add two drops of ProtoSlo. Using a toothpick, ***gently*** stir the water and the ProtoSlo together.

3) Place the cover slip on top of the slide.

4) View the slide at 4X using the steps 1 – 8 on page 2. Scan around on the slide until you find a moving microorganism.

5) If the microorganism is not swimming quickly, try to observe it under 10X power. Recall, you do this by bringing the specimen to the exact center of your field of view. Now switch to the 10X lens. You may have to focus using the fine focus knob.

6) Sketch the microorganism in the circle on the right. Next to your sketch write the total magnification that you are using.

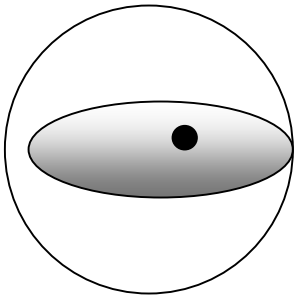


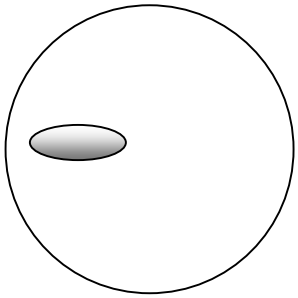
7) For now, set the slide aside. You will view it again in the next section.

j) Estimating the size of a specimen

The microscope not only magnifies a specimen but it also can be used to estimate the actual size of the specimen in millimeters.

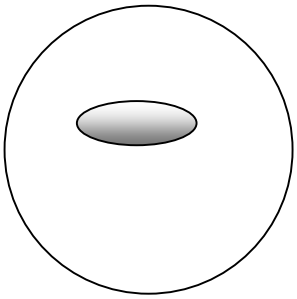
The big circle on the right represents the field of view on the 10X lens. The oval shape is a cell that you are viewing. You might want to know how long the cell is, in millimeters. How can you find the size of the cell? Suppose that you found the field of view of the 10X lens is about 1.5 millimeters in diameter. Since this cell spans almost the entire field of view, the cell must be almost 1.5 mm, (perhaps 1.4 or 1.3 mm).



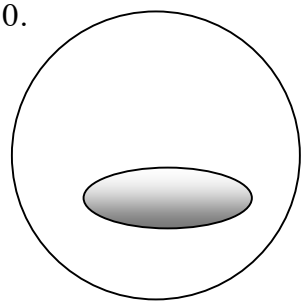


In a similar way, the cell on the left takes up about a third of the field of view, so on the 10X lens, this cell must be about one third of 1.5 mm, which is 0.5 mm.

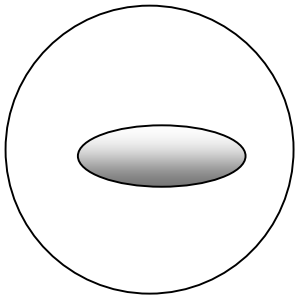
If you were observing the cell on the right using the 40X lens, the cell's size must be about 0.2 mm. This is because it takes up about half of the 40X field of view, which is about 0.4 mm.



Estimate the sizes of the two cells below using <u>your own</u> field of view numbers from the table on page 10.



Viewed using 10X lens
Size: _____ mm



Viewed using 40X lens
Size: _____ mm

Now go back to the slides of your cheek cell and your microorganism. Make an estimation of their sizes and add your estimations to your sketches on pages 6 and 7.

Clean up: No glass in trash! Wash off the two wetmount slides with lots of soap and de-ionized water, then dry them and then put them back in their box. The plastic cover slips go into the trash, not into the glass waste.

k) The dissecting microscope

The microscope you have used so far in this exercise is called the compound microscope. Another type of microscope that is frequently used in laboratories is the dissecting microscope.

Although dissecting microscope does not have as high a magnification as the compound microscope, the dissecting microscope does have several advantages: (1) It can be used to view whole specimens (whereas the compound microscope requires that a thin slice of the specimen be prepared). (2) The dissecting does not require that the specimen be mounted onto a microscope slide. (3) The dissecting microscope is usually easier to focus than the compound microscope.

Take the dissecting microscope out from your cabinet and place it on your lab bench. Follow the procedure below to familiarize yourself with the dissecting microscope.

a) Exit the lab room and collect an outdoor specimen to view. The specimen can be anything you choose, such as a leaf, a mushroom, an insect or worm.

b) Place the specimen under the dissecting microscope. If your specimen is moving, place it in a glass dish to keep it crawling away.

c) Turn on the illuminator (a flashlight-like device) and adjust its angle so that it shines on the specimen.

d) Locate the magnification adjustment knob at the top of the microscope. Set the magnification for as low a possible to start with. Look through the ocular lenses and adjust the focus (focus knob is on the side of the microscope).

e) Use the magnification adjustment knob to zoom in on any areas of the specimen that look interesting to you.

f) Sketch your specimen on the back of this page. Show the sketch to your instructor.

g) If your specimen was an animal, please set it free when done viewing.

l) Results table:

Objective Lens	Field of View size	Total Magnification	Brightness
4X	_____ mm	_____ X	
10X	_____ mm	_____ X	Dimmer/Brighter (circle one) than 4X
20X	_____ mm	_____ X	Dimmer/Brighter (circle one) than 10X
40X	_____ mm	_____ X	Dimmer/Brighter (circle one) than 20X

m) Review questions:

1) What is the total magnification when using the 40X lens? _____

2) If the eyepiece lenses were changed to 4X magnification, what would be the total magnification using the 4X objective lens? _____

3) The term for a slide you prepare yourself by putting the specimen in a drop of water: _____

4) Write 4X, 10X, 20X, or 40X in the blank after each description that matches it. Some descriptions may require more than one answer.

a) It is sometimes called the Low Power lens: _____
b) You should only use the fine focus (not use the course focus) while using this lens: _____
c) It is sometimes called the Scanning lens: _____
d) It gives the dimmest image of the four lenses: _____
e) It is an objective lens: _____
f) It gives the brightest image of the four lenses: _____
g) It has the smallest field of view of the four lenses: _____
h) When using this lens, the microscope has a total magnification of 100X: _____
i) It is sometimes called the medium power lens: _____

5) The term for a slide that is made for you (usually by a scientific supply company) _____

6) The term for the small glass square that goes on top of the specimen: _____

7) Give the term for the part of the microscope that…

a) The slide clips into: _____

b) Is the flat surface that the slide rests on: _____

c) Adjusts the brightness of the image while you are viewing the specimen: _____

d) The knobs that move the slide left, right, up, and down: _____

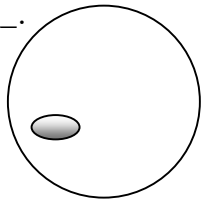
e) Makes <u>small</u> changes in focus: _____

f) Makes <u>large</u> changes in focus: _____

g) The lens you look into: _____

8) In the margin, draw what the letter "h" would look like under the microscope.

9) If you move the slide to the left, the image will move to the _____.



10) Estimate the size of the cell shown to the right, when viewed using the 40X lens: _____ mm

11) What does ProtoSlo do?

12) What are three advantages of the dissecting microscope compared to the compound microscope? What is the dissecting microscope's main disadvantage compared to the compound microscope?

m) Review questions answers:

1) 400X

2) 16X

3) Wet mount slide

4)
   a) 10X
   b) 40X
   c) 4X
   d) 40X
   e) 4X, 10X, 20X, 40X
   f) 4X
   g) 40X
   h) 10X
   i) 20X

5) Prepared slide

6) Cover slip

7) Give the term for the part of the microscope that…
   a) Slide carrier
   b) Stage
   c) Condenser Iris Diaphragm
   d) Mechanical stage knobs
   e) Fine focus
   f) Course focus
   g) Ocular lens

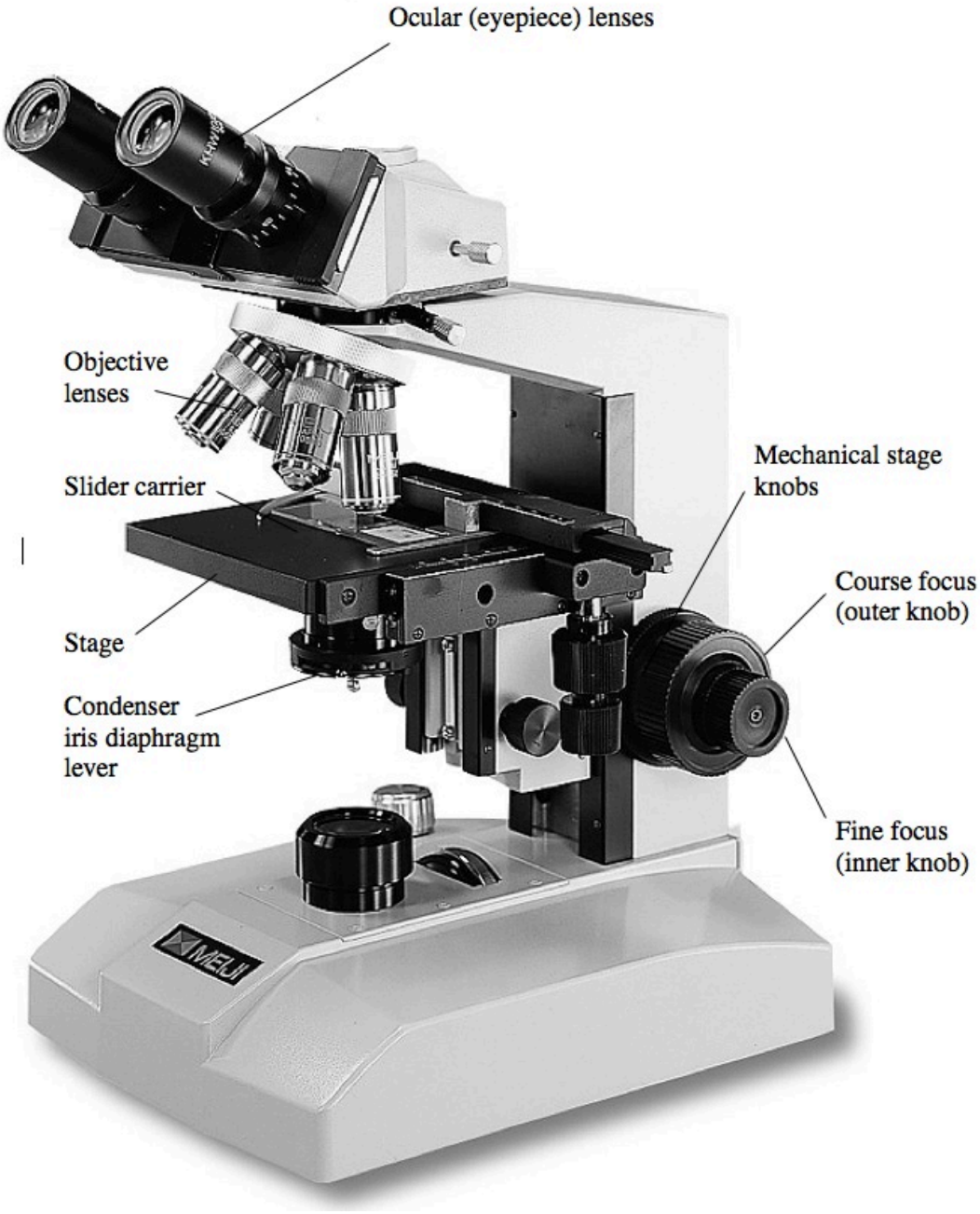
8) Draw what the letter "h" would look like under the microscope: 

9) Right.

10) Roughly 0.1 mm

11) Protoslo slows down the movement of microscopic organisms, making them easier to view under the microscope.

11) Advantages = Can view whole specimens, Specimen does not have to be mounted on a slide, and Easier to focus. Disadvantage = Magnification is not as high.


Ocular (eyepiece) lenses
Objective lenses
Slider carrier
Stage
Condenser iris diaphragm lever
Mechanical stage knobs
Course focus (outer knob)
Fine focus (inner knob)
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