Department of Biological Sciences

University of Rhode Island

Principles of Biology – Bio 101

Laboratory Manual

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Welcome to Bio 101 Lab!

Lab structure and objectives:

Laboratory sections in Bio 101 are small to provide you with a direct, interactive experience with a number of common biological fields and methods. The labs will follow the subjects introduced in lecture, and are designed to allow you to experience these topics. Because your participation is an important part of your Biology experience, the lab portion of Bio 101 is worth 25% of your overall Bio 101 grade. Many students find that with effort and attendance at every lab, the laboratory's hands-on experience will positively affect their biological learning experience and their overall Bio101 grade.

Lab expectations

Be prepared to learn.

Most students feel they learn best in an active, hands-on environment, and this is it for you! Come to lab having read over the lab so that you are ready for what your Teaching Assistant (TA) will outline for you to do. The labs are designed so that you should be able to complete each lab on your own, with your TA offering some assistance. Some of the labs will be demonstration/ concept review labs in which you will learn or review biological techniques and/or equipment use. Other labs will be experimental in which you will collect data, then analyze and present findings. Overall, the Bio101 labs are designed to help you with the lecture material, to give you some experience with different biological equipment and techniques, as well as give you a taste of what a few fields of biology are like.

Be on time.

Most of the exercises require the full lab period (1 hour, 50 min). Important information will be given out at the beginning of lab. The weekly quiz will be given and completed within the first 15 minutes of class.

Have the lab manual and be prepared for lab. Consider purchasing the recommended text.

Bound lab manuals are available in the school bookstore.
Online lab manual is available at: www.uri.edu/cels/bio/wetherbee/bio101/lab.htm or on Sakai.
Each student must bring their own copy of the lab instructions to lab room, or lose 10% on the quiz that week. Bring either a printed copy or your bound lab manual with you.

Recommended text:

Although not required, "**A Short Guide to Writing about Biology" by Jan Pechenik** is recommended. We will reference the 7th edition, but earlier additions will also help you (and cost less). This guide will improve both your scientific writing skills and your understanding of presenting information scientifically. There will be copies available on reserve in the library. However, if biology is your major, we recommend you purchase this book

Hand in assignments on time, in hard copy format.

➔ Late assignments will be graded at 50% of the original points and will only be accepted up to midnight of the day the assignment is due. Hand assignments in during lab to be on time. For late assignments, send your TA an email with your work attached before midnight the day it is due. Bring a hard copy to lab the following week.

Class work must be completed and handed in before leaving the laboratory.
➔Students must hand in assignments on paper (hard copies).

Observe all safety rules.
NO food or drink allowed in the lab EVER.
Cell phones must be off or silent during lab: no ringing, no talking, no texting.
If you receive an emergency call, please leave the room to answer.
Wear closed-toe shoes to protect yourself. Use caution with any potentially hazardous objects (razor blades, broken glass, toxic chemicals). Notify your TA immediately if there is any injury or hazardous activity.
Those students not following safety rules will have to leave the classroom.

Maintain academic honesty.
The University of Rhode Island and the URI Biological Sciences Department are committed to maintaining a high academic standard. We strive to insure that all students earn their grades with their own work. Cheating diminishes the work and degrees of honest students, and so cheating will not be tolerated.

Cheating is defined in the URI student manual (http://www.uri.edu/facsen/8.20-8.27.html).
Cheating includes:
— Any quiz or assignment that is plagiarized *in whole or in part.*
— Work submitted by students that are identical *or nearly identical.*
(Please be aware that students are encouraged to study and review lab work in groups; however, all students must hand in their own written assignments to receive credit.)

There are serious consequences for students found cheating.
1) TA will give student(s) a zero for that assignment or quiz.
2) Copies of the work and/or the TA's description of cheating will be submitted to the dean of the student's college, and to the University Board on Student Conduct.
3) Copies of the work and/or the TA's description of cheating will be placed in a file in the Office of Student Life.
4) Subsequent infractions (cheating again in that student's college career) will be noted by the Dean of Students who may initiate conduct action again the student. These actions may be serious (as the case warrants) and may include dismissal from the university.

Contacting your TA: email

Your TA will give you their email address on their course syllabus the first day of lab. This is your best way to contact your TA, whether to deal with situation through email or to set up an appointment to speak with your TA in person.
Please read the following page of tips on how to write a professional email.

If you have problems contacting your TA, you may contact the Bio101 lab coordinator, Linda Forrester at <lindaforrester@uri.edu>

Note: Students with special requirements should discuss their situation with their TA.

Tips on how to write good, professional email

This list is an amended version of a list written by Todd Smith, founder of the website Little Things Matter. Please use these comments when writing professional emails.

1. **Take pride in your emails** — Every email you send makes an impression and plays a small role in defining you. What you say and how you say it will play a significant role in how you are viewed.
2. **Select an email address that identifies you—**Preferably, select an email address that includes your first and last name. This will make it easier for people to identify you by your email.
3. **Be friendly—**Your demeanor in your online communication should be similar to how you interact offline. Take the extra time to type something friendly at the beginning and/or at the end of the email. It could be simple one-liners such as "I hope you had a nice weekend" or "Thanks for your help."
4. **Be professional—**If you want to be viewed as a serious student, then make sure you present yourself as one.
5. **Proof your emails—**Never send an email without proofing it at least once. If it is important, then read it two or three times to make sure you are proud of what you have written. Look for missing words and misspellings.
6. **Don't use text lingo—**Emails not text messages. Spell things out.
7. **Address the person to whom you are writing. (Use their name.)** — Type out the name of your Teaching Assistant (TA) or Professor. Your letter should look like you wrote it specifically to that one person. You may address the person teaching your class as "Professor __X__" unless you have been given permission to be less formal.
   It is also nice to say "Hi" or "Hello" or "Good Morning" instead of "Yo, Doc."
8. **Summarize your email in the subject line—**Take the time to summarize the subject of your email in a few short words.
9. **Put your name at the end of your emails—** ***Always*** sign your name. Use your full name or add a signature with your full name at the end of your email.

Microscopy
Compound and Dissecting Microscopes

<u>**Learning objectives for lab**</u>

Be able to identify parts (and their function) of dissecting and compound microscopes.

Be familiar with basic skills of microscopy, including:

-Explain how to properly handle the microscope.
-Explain how to focus the image.
-Explain how to change light intensity and light focus.
-Determine the magnification and size of the field of view of the microscope.
-Determine depth of field.

Begin to learn & utilize basic scientific drawing and writing descriptive text.

History / Background

Biologists use many tools in the laboratory, including microscopes in various. Two types of microscopes that you will use frequently in this laboratory are the compound and dissecting light microscopes. Of these two, the compound microscopes we use have more adjustments available to you, and so may be more challenging to use. Successful use of any microscope depends on a variety of factors including quality of slide preparation, proper focusing, and adjustment for optimal illumination. A less difficult but no less important tool is the micropipette, critical for delivering accurate volumes of liquids. You will learn to use micropipettes later in the semester.

A **simple microscope** is nothing more than a single convex lens, also called a magnifying glass. A **convex lens** is one in which the center of the piece of glass curves out (fattest in the middle). The other major type of lens is the **concave lens**. It is a piece of glass that is thickest on the edges and curves inward in the middle. Looking at it on its side, it forms two "caves". Concave and convex lenses can be used in combination to produce various optical effects. A **compound microscope** is one that uses two or more lenses together.

Compound microscopes (that is, microscopes using more than one lens in succession) were invented around the 1590s by **Zaccharias Janssen** and his son **Hans** (from Holland). **Robert Hooke** (England) built the first useable British compound microscope in about 1655. Using his microscopes looking at a slice of cork, he described "cells", like the tiny rooms used by monks, coining the term that we still use today. Although he worked after the beginning of the compound microscopes, **Anton van Leeuwenhoek** (*layu-wen-hook*) (Holland) built many superior **simple microscopes** (having only one lens) that magnified items over 200 times, starting about 1670. His tiny lenses had incredibly good resolution, and allowed for many new discoveries. Because of his many discoveries, he is often known as the **"father of microscopy."** He described in 1674, the green alga Spirogyra, in 1683 living bacteria, in 1702 the ciliate Vorticella. He also was first to describe microscopic sperm, foraminifera, nematodes and rotifers.

Some compound microscopes have only a single viewing ocular. These are called **monocular scopes**. In this lab we will utilize **binocular** compound microscopes, using two oculars. Modern compound microscopes are designed to give a magnified two-dimensional image that can be examined on different focal planes.

Stereomicroscopes or **dissecting microscopes** are designed to give a third dimension to viewing, depth. Each eyepiece uses a separate lens to capture an image from a slightly different angle from the common main objective. The stereomicroscope takes advantage of our brain's ability to perceive depth by transmitting slightly different angled views of an object to obtain a three-

dimensional image, the same way human eyes work together to obtain depth perception. The ocular lens then multiplies, or *"compounds,"* the objective magnification in the stereo microscope in the same manner as it does in the *"compound microscope."*

Stereomicroscopes were invented about the same time as compound microscopes, however only in the 1890s, with advances by Horatio Greenough, did the stereomicroscope become a workhorse in the scientific lab. Stereomicroscopes are now (with compound microscopes) commonly used in most biological labs. They are especially useful for viewing opaque objects at low magnifications or when manipulating specimen. They are commonly used for dissections, surgeries, and for micro-electronics. In order to perceive the 3D effects, both eyepieces must be utilized.

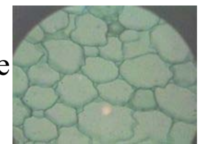
Ultimately, this lab is designed to teach you how to use the advanced properties of microscopes properly, so that you can become familiar and skilled at microscope use, because microscopes are so important in most fields of biology, and their proper use will make many biological field so much more exciting and available to you. **Specifically**, you should learn all the parts of the microscope, how to properly use all the parts of the scope. You will spend some time learning about the two main types of scopes used in biology, looking at a variety of samples through the scopes.

Microscope terms

Magnification A light microscope allows the user to enlarge the image (magnification) of the object being studied and to observe details of structure not detectable with one's unaided eye (resolution) especially if the light is adjusted properly.

The image of the specimen under the microscope is formed by the magnified image that passes from the objective lens that is then magnified again by the ocular lens. Magnification is the product of the magnifying power of the objective lens times the magnifying power of the ocular lens. Example: If the ocular lenses are 10X, the magnification of a specimen using the 10X objective would be 10X times 10X, or 100X.

Total magnification = magnification by ocular lens X magnification by objective lens

Observe plant stem with:
4x objective 10x objective 40x objective 

Using 10x ocular
Total mag = 40x 100x 400x

Field of View **The** field of view refers to the area that you can see through the oculars. Your field of view (area you see) gets smaller with increasing magnification. In the pictures above, you can see the entire cross section of the plant stem with the lowest power objective, but with the 40x objective you can only see cells in the center of the stem cross section (so, a smaller field of view).

Depth of Field The depth of field refers to how much of the depth of your view is in focus. Even in thinly sliced tissues on slides, you will be able to see different levels of three dimensional objects through your microscope. With a narrow depth of field, you can use the fine focus to examine the top, middle or bottom of specimens. With a wide depth of field, you can see the entire specimen from top to bottom, but it may not be as sharply in focus.
You can increase your depth of field (depth of view in focus) by closing the aperture iris diaphragm,

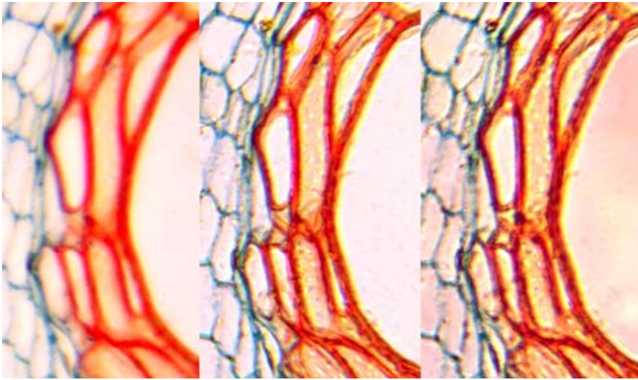
and conversely, have a narrow depth of field by opening the aperture.

Kohler Illumination **To** have the highest and most uniform light (given that we have a non-uniform light source, a light bulb), you must use "Kohler illumination." In 1893, August Kohler described a method of setting up a compound light microscope to achieve the most uniform illumination from a non-uniform light source such as a light bulb. The result has become known as Kohler illumination and is obtained:

(A) by focusing the bulb filaments at the plane of the condenser diaphragm (which is already done in scopes with built in lights)

(B) by focusing an image of the lamp diaphragm at the plane of the object. YOU will do this!!

To obtain Kohler illumination, you adjust both 1)) the opening of the aperture of the condenser iris, by using the aperture iris diaphragm lever, and 2 the height of the condenser by adjusting the condenser height adjustment knob. (Instructions follow in "advanced setup of microscope.")

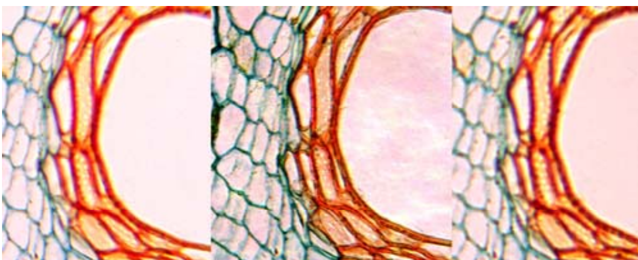


How the aperture opening affects the image.

The first picture shows the aperture open all the way, allowing light to scatter, reducing details and contrast.

The second picture shows the aperture open the correct amount.

The third picture shows the aperture closed too much, so the cone of light does not fill the view of the objective lens. So the contrast has increased but the resolution for details is worse.



How the condenser focus affects the image.

For the first picture, the condenser was as close to the slide as possible. For the third picture, the condenser was as far from the slide as possible. The center picture shows the condenser in its optimal position for the best resolution.

Resolving Power and Useful Magnification Microscopes are designed to produce an enlarged, focusable image of a specimen. Images can be made larger many times, as you have experience with taking a digital photo and enlarging it many times. However, you have to realize at some point, making an image larger eventually stops being useful. The basic limitation of enlarging any image is its resolution. If an image is enlarged to a point that it becomes blurry or indistinct, then further magnification does not help. You need to improve your resolving power.

The resolving power of a light microscope is a measure of its ability to distinguish detail in a specimen. Resolving power is expressed as the limit of resolution (LR).

The limit of resolution (LR) is the smallest distance by which two neighboring points can be separated and still be seen as separate entities. The limit of resolution of your eye is between 0.1 and 0.2 mm. That of a good quality light microscope is about 0.2 µm (1 µm = 0.001 mm), a 500-1000 fold improvement over your eye.

You can alter the resolution of your image in several ways, but you want to have high contrast in order to have the best resolution. Contrast can be changed by staining samples with artificial dyes to make different tissues more visible. You will see this in some of the prepared slides we examine today, as well as later in the semester when we stain our own slides to improve contrast. You can also change the aperture iris diaphragm (contrast control). When the diaphragm is stopped down (nearly closed) the light comes straight up through the center of the condenser lens and contrast is high. When the diaphragm is wide open the image is brighter and contrast is low. You will need to find a balance between having the image bright enough to allow contrast and restricted enough to increase contrast.

There is not one set rule to follow for light adjustment. You will learn over time to adjust your lighting as you move between different magnification levels, and between specimens of different densities, thicknesses and contrasts.

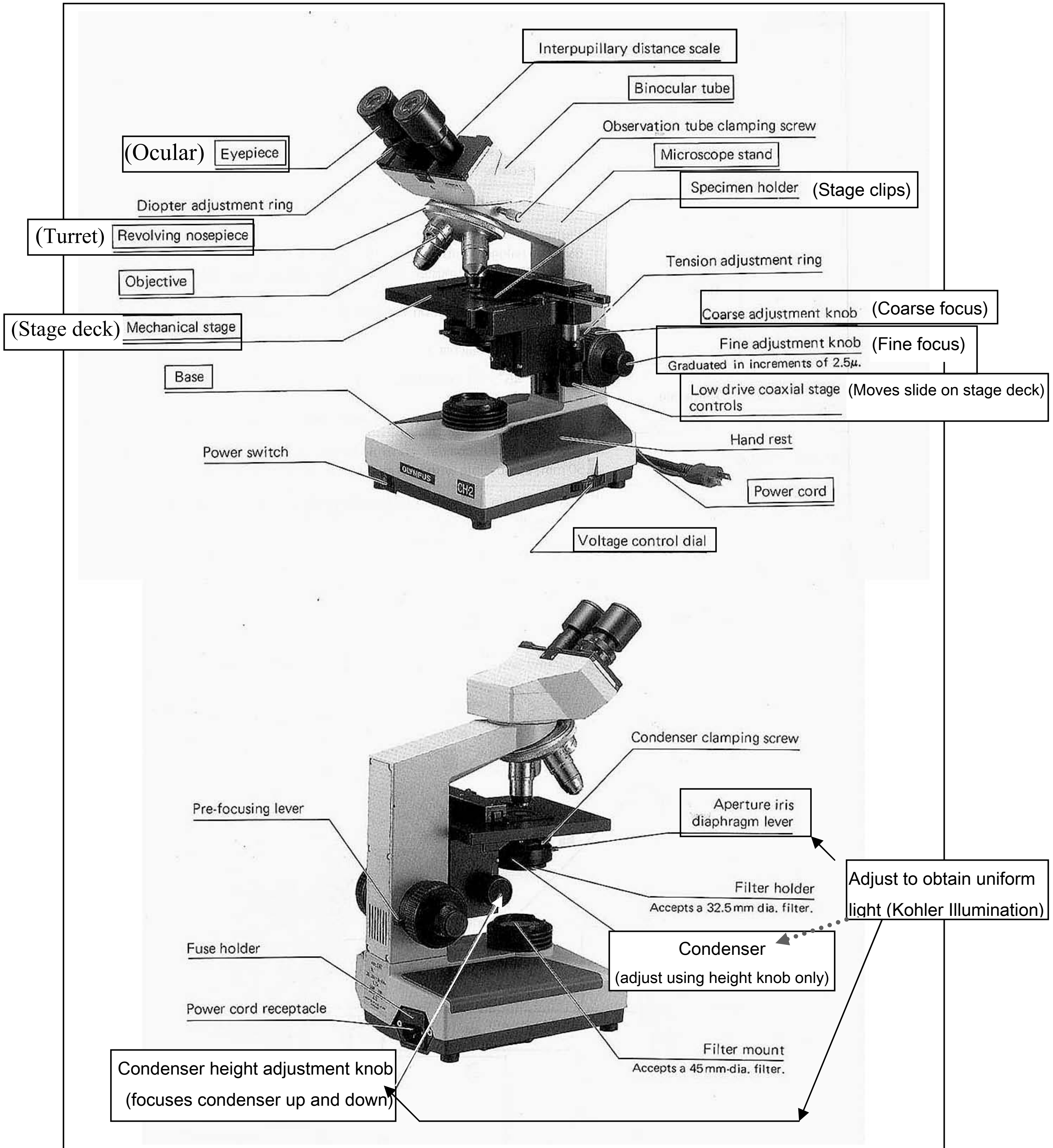
Dry and Oil-Immersion Lenses Compound light microscopes often have 4 objective lenses (4X, 10X, 40X, and 100X). The 4X, 10X and 40X objectives are called 'dry lenses' because they are designed to work in air. The 100X objective is an oil-immersion lens because its front element must be submerged in a drop of oil over the specimen. (We will not be using the oil-immersion objective in this class.)

Using a light microscope – General Precautions

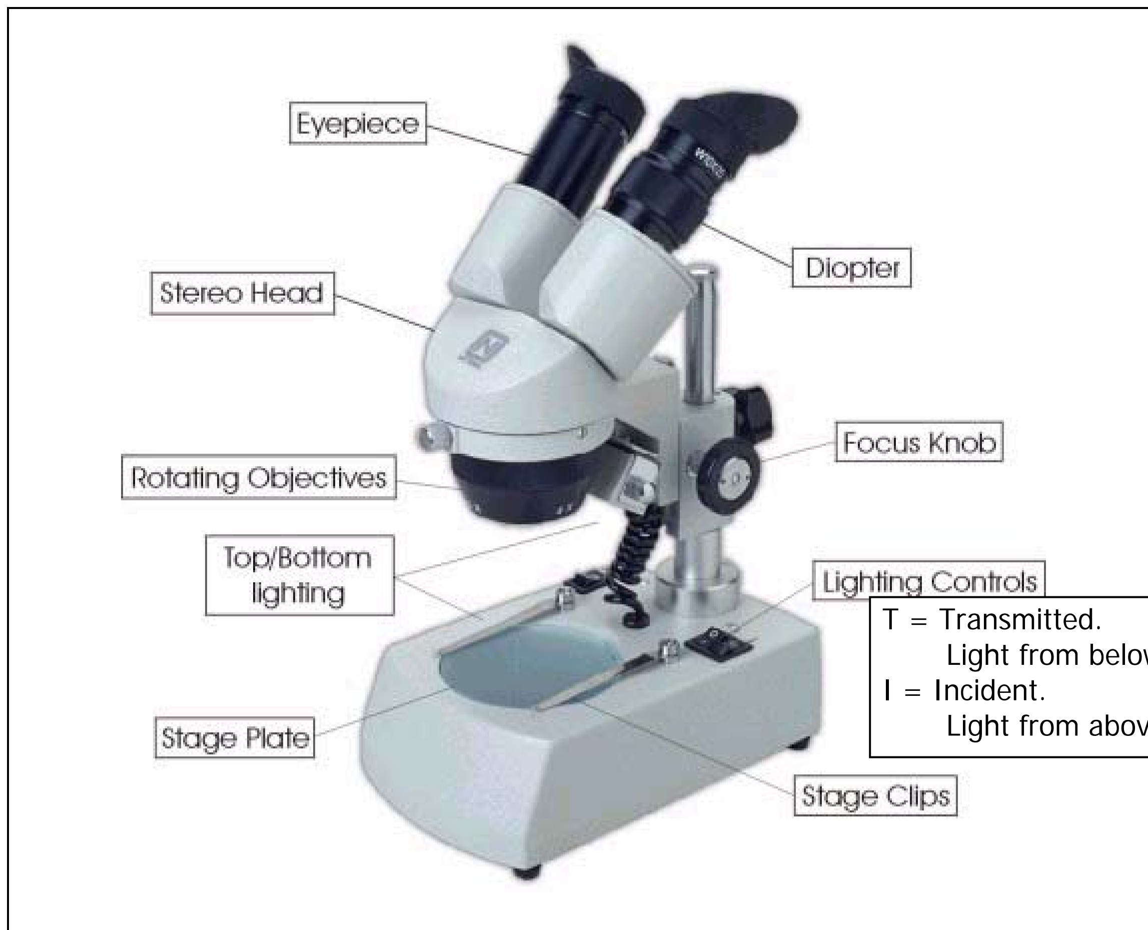
1) **Carry the microscope by its arm with your other hand supporting the base.** DO NOT TILT the microscope when carrying it. The ocular lenses may fall out.
2) Do not remove objective lenses or take them apart.
3) Clean the surfaces of lenses **ONLY** with lens paper.
4) Always focus **upward** when your eye is at the eyepiece and you are using the coarse focus knob.
5) When rotating objectives, be sure they do not contact the stage or slide.
6) When using a compound microscope, always keep both eyes open. Move the ocular lenses to make it possible for you to look through both lenses, and life with a microscope will improve greatly!

NOTE: The 100X objective lens can only be used when it is immersed in special oil.

The Olympus CH-2: <u>Compound Light Microscope</u>



National: <u>Stereo (Dissecting) Microscope</u>



T = Transmitted.
Light from below.
I = Incident.
Light from above.

Student Procedure for lab

Basic setup of microscope

1) Check that the intensity is set below 5. Turn on the power to the light.
2) Using **coarse focusing knob**, lower the **stage deck** to the lowest position.
3) Place a prepared slide on stage deck, using **stage clip**. Move the slide into position using the adjustments available for the **mechanical stage**.
4) Turn the **turret** of the microscope so that the **10x objective** is pointing at the object. Always begin observations using the 4X or 10X objective lens.
5) With your head to the side and your eyes level with the stage, use the coarse focusing knob again. Raise the stage until it is as close to the object/slide as possible.
   Do not ever force the knob. Do not ever let the slide touch the objective.
6) Rough focus… Look through the oculars. Slowly LOWER the stage with the coarse focusing knob until you see an image jump into sharpness. Fine tune the image with your **fine-focusing knob** until it is as sharp an image as possible. NOTE: The fine adjustment has a limited range through which it operates. Its movement may stop at either end of the range. You can reset the fine adjustment near the middle of its range by adjusting the coarse focus knob.
7) Adjusting the **oculars** to your specific eye ability… Close your left eye. Using only your right eye, fine tune the image with your fine-focusing knob until it is as sharp an image as possible. Open your left eye and close right eye. Look at the image through the left ocular lens with your left eye. *Do not touch the coarse or fine focus.* Rotate the **diopter adjustment ring** at the base of the left ocular lens to focus the image of the specimen without adjusting the focus knobs. This scope is now set for your eyes' abilities.
8) Adjusting the oculars to your specific eye width… Adjust the ocular lenses for the distance between your eyes so that perfect binocular vision (a single, circular field when viewed with both eyes) is obtained. Adjust the **interpupillary distance** scale to do this. You do this by gently pushing/pulling the oculars closer together or further apart.

Advanced setup of microscope

1) Focusing the light for Kohler Illumination… Start with the **condenser** rotated up as close to the stage deck as possible; use the **condenser adjusting knob** to move it up to the stage deck. Start with the **iris aperture diaphragm** stopped down (high contrast). "Stopped down" means that the iris aperture is nearly closed. You should see the light that comes up through the specimen change brightness as you move the aperture diaphragm lever. Watch the changes in the depth of field and the amount of the image in that is in focus. When the image is best lit for your purpose, either with great depth of field or extra fine focus on a single plane, then stop adjustments.

2) Adjusting the brightness of the image… Use the **lamp brightness adjusting wheel** on the side of the **base** of the scope. Do not use the condenser or the iris diaphragm to change the brightness as this will change your light focus.

To TURN OFF microscope

1) Turn down the voltage to the light (to about "3").
2) Turn off light.
3) Turn turret so a low power objective is nearest slide.

Summary of COMPOUND MICROSCOPE information

To change:	Adjust:
Position of object in view	Low drive coaxial stage controls (aka: the x- and y- controls)
Magnification	Turret, to change objective
Focus on image	1) coarse adjustment knob 2) fine adjustment knob 3) eyepieces, check they are focused for your eyes 4) focus of light
Field of view	Turret, to change objective Higher magnification, smaller field of view
Depth of field	Iris aperture diaphragm Closing iris diaphragm, increases depth of field (but decreases amount of overall light to image).
Kohler illumination (to focus the light)	Iris aperture diaphragm & Condenser height
Resolving power (contrast control)	Iris aperture diaphragm Closing iris diaphragm causes light to be more unidirectional, increases contrast (but decreases amount of overall light to image).

Summary of STEREO / DISSECTING MICROSCOPE information

To change:	Adjust:
Position of object in view	Move with hands on stage
Magnification	Zoom knob
Focus on image	1) focus knob 2) check eyepieces are focused for your eyes
Field of view	Zoom knob Higher magnification, smaller field of view

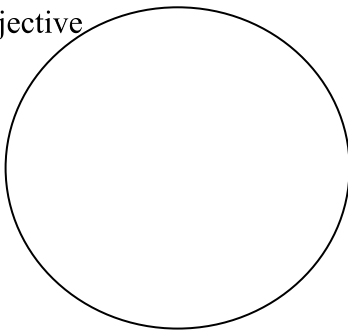
Procedure for today's lab

First, follow the preceding procedures to learn to focus the image and the light for your microscope. You should be able to show your TA that you can use the rough and fine focus, focus the oculars to your eyes, change the interpupillary distance to your eyes, focus the light with the condenser and iris aperture diaphragm, and change the brightness of light. **Then**, continue with the following exercises.

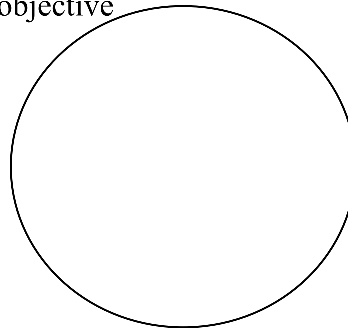
Exercise 1: You will start with a prepared slide with the letter **e**. Place the e on your stage deck so that the e is oriented so that you can read it. Center the e in your field of view.

Draw a picture of exactly what you see using the 4x, 10x and 40x objectives. The image should remain centered and roughly in focus, as most light microscopes are **parcentral** and **parfocal**, meaning the image will remain (parcentral) centered and (parfocal) nearly focused when changing from one objective to the others. Fine focus adjustments should be all that is needed.

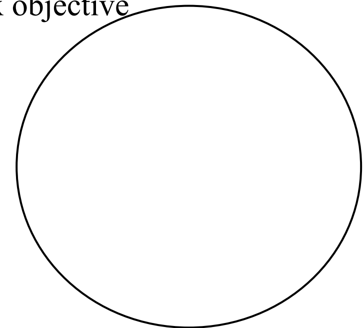
4x objective



10x objective

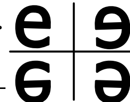


40x objective



Total magnification = ________ Total magnification = _________ Total magnification = ________

Looking at the side of the scope, using the coaxial stage controls (the X – Y controls) move the slide to your right. Now do this while looking through the scope.



How is the e oriented? Describe the orientation __________________

Which way does the image move when you move the slide to the right?? __________________

Determine the total magnification of the image using each objective.

Which objective provides the largest field of view? ______________________________

Which objective should you use to begin examining a specimen? ________________________

Why is it difficult to locate an object starting with a higher-powered objective? ____________
____

Exercise 2. Change to a prepared slide of three colored threads. Examine the threads using the low-powered objective. Focus up and down to determine which thread is on top and which is on the bottom in one area. Use the condenser and iris aperture adjustments to change your depth of field.

Can you have all three threads in focus? ____

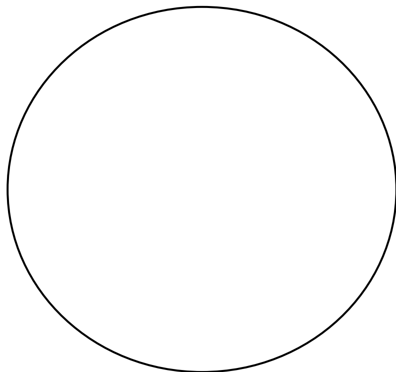
Re-examine the thread under high powered objective.

Are all three threads in sharp focus simultaneously using the high-powered objective? _____

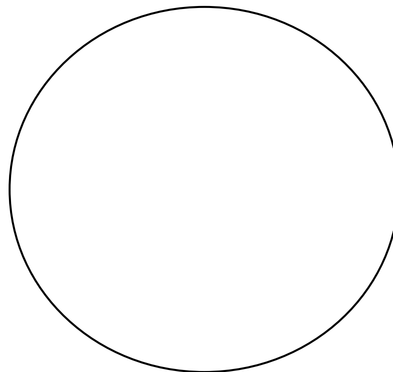
Which objective has the greatest depth of field? ___________________

Exercise 3. Comparing YOUR hair to dog hair.

Prepare a slide with a piece of your own hair. Compare to one of the pieces of dog hair from the front bench. Examine the hair under the dissecting and compound microscopes. Draw what you see.



Dissecting scope image



Compound scope image. Total mag= _______

Exercise 4..

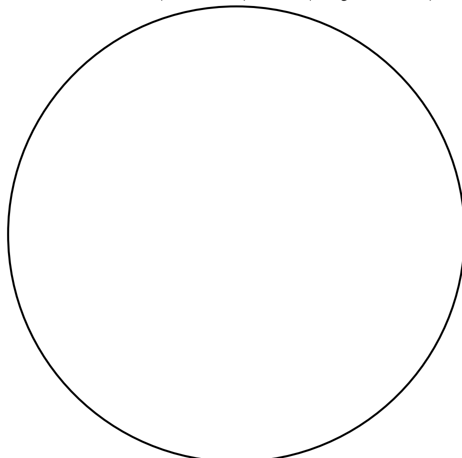
You will examine **three different slides of micro-organisms- some alive and some prepared.** Look at the slides using all three objectives. Draw the micro-organism using the magnification that is most appropriate (showing the entire organism up close and with good detail).

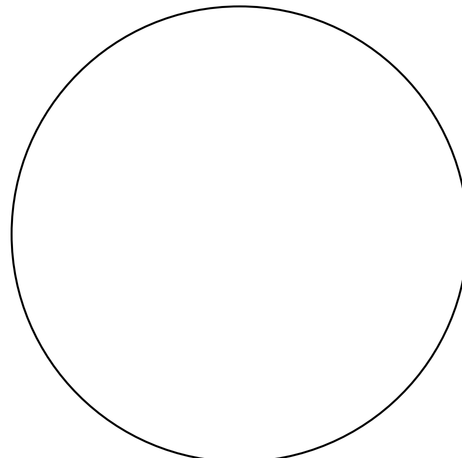
You will examine **one live slide of *Daphnia* that you will prepare**. *Daphnia* is a living freshwater pond organism that we will utilize later this semester to test its physiology. Today you will prepare a slide of *Daphnia* and examine it using the dissecting scope. Draw *Daphnia* using the magnification that is most appropriate (showing the entire organism up close and with good detail).

Be sure to show your drawings to your TA. Your TA is not judging you on your ability to draw (although that helps!) but on your care and attention to details. Remember, all the labs in this class are designed to help you to help yourself to learn. Put forth the effort that helps you improve your abilities.

Micro-organism – Slide 1 Organism name: ___________________________

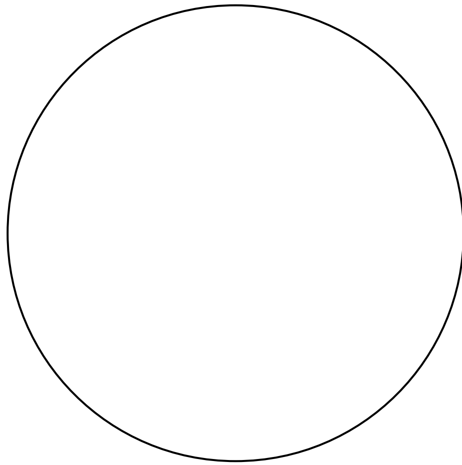
Magnification = ______X _______ =_________ Magnification =___________
(ocular) X (objective)= total mag

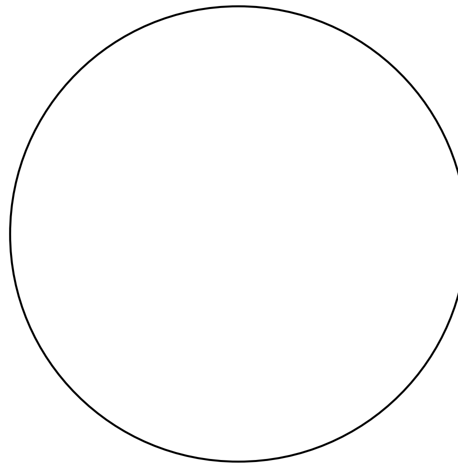




Micro-organism – Slide 2 Organism name: ___________________________

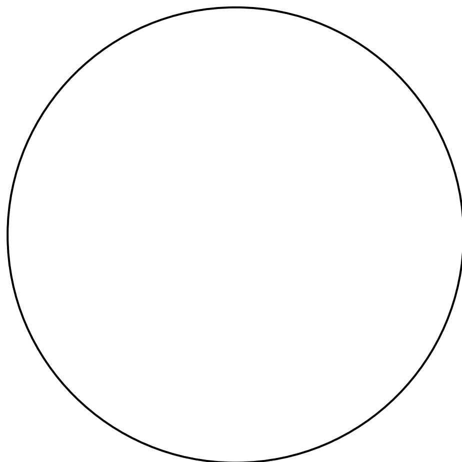
Magnification = ______X _______ =_________ Magnification =___________
(ocular) X (objective)= total mag

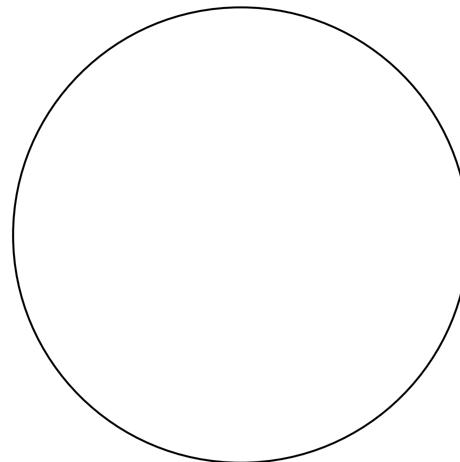




Micro-organism – Slide 3 Organism name: ___________________________

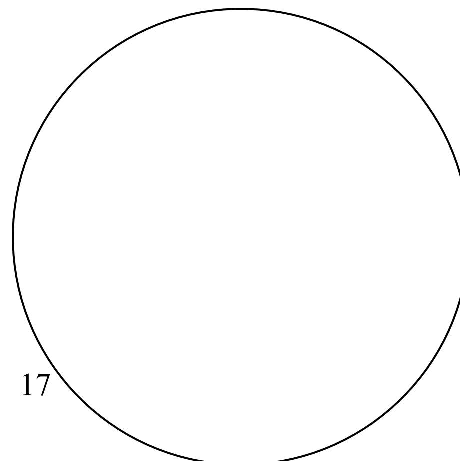
Magnification =_________ Magnification =___________





Live Daphnia slide – *Use Dissecting scope*

Magnification =_________



Vocabulary (be prepared to identify, describe, and/or know how to adjust these)

- simple microscope
- compound microscope
- stereo microscope (dissecting microscope)
- convex lens
- concave lens
- Zaccharias and Hans Janssen
- Robert Hooke
- Anton van Leeuwenhoek
- monocular
- binocular
- magnification
- field of view
- depth of field
- resolving power
- dry lens vs. oil immersion lens
- the ocular lenses / eyepiece
- interpupillary distance scale
- the objective lenses / objectives
- turret /revolving nosepiece
- arm / microscope stand
- foot
- stage deck/ stage plate
- stage clips
- coarse and fine adjustment knobs
- diopter adjustment ring
- mechanical stage
- low drive coaxial stage controls (X and Y controls)
- condenser
- condenser height adjustment knob
- aperture iris diaphragm
- built-in light source
- voltage control knob for light intensity (lamp voltage slider)
- parcentral
- parfocal

Lab Practical

Assessing basic practical laboratory skills

Objectives

1. Students will demonstrate ability to use a balance and micropipette.
2. Students will demonstrate ability to use microcentrifuge.
3. Students will demonstrate ability to use both compound and stereo-zoom (dissecting) microscopes.

Background information

There are basic skills expected of all students before finishing Biology 101. We will test you on a few of the most essential and easy to demonstrate in a lab setting. This lab practical will be 10% of your final lab grade.

Students will have time to practice their skills during the lab periods prior to the practical. Students will only be allowed to take the practical one time only. Students must be prepared to take practical during their lab time. Your TA will tell you the exact date of your lab practical.

Skills to be exhibited by students

1. Demonstrate how to turn on the balance. (0.5pt)
   Show how to zero out a weigh boat. (0.5pt)
2. Demonstrate how to use a micropipette by dispensing the correct mL of water onto the balance. (For example, you might be told to get a water sample weighing 0.750g.)
   — Show how to set the pipette to the correct volume (1pt).
   — Show how to pipette that volume correctly (1pt).
3. Demonstrate how to spin a single microtube sample in a microcentrifuge.
   (There will be balance tubes available to you for your use.) (1pt)
4. Describe briefly to your TA the parts of the compound microscope needed to focus the light on the image. (1pt)
5. Using the compound microscope, demonstrate how to place slide in view, with the requested part of the slide in the center of the view, under high power. (1pt)
   Demonstrate focusing both oculars to student's own eyes, changing the diopter distance to fit student's eyes. (1pt)
6. Using the stereo-zoom (dissecting) microscope, demonstrate how to place an object in view, with the requested part of the object in the center of the view, under high power. (1pt)
   Demonstrate focusing both oculars to student's own eyes, changing the diopter distance to fit student's eyes. (1pt)
7. Demonstrate the correct way to turn off compound and dissecting scopes. (1pt)