# BI 122: Biology Lab series
# (Microscopy module)

**Juniata College**
**Department of Biology**

Using the *Caenorhabditis elegans* model organism to study animal physiology, learn microscopy techniques, and practice scientific inquiry

## Table of Contents

# BI122 Microscopy

## i. Overview of the course

Supplemental syllabus for the Microscopy Module: Spring 2017
Other general course procedures, grading, and rules may be in a BI122 syllabus (See Dr. Grant).


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**Overview.** In this lab module, you will learn how scientists perform experiments. Specifically, we will focus on how a chemical (ethanol) affects an animals' behavior. While performing these experiments, we will learn about scientific inquiry and basic microscopy techniques. Using those techniques, you will be given the opportunity to be a scientist by developing a research question of your own, designing the experimental plan, collecting data, and presenting the results. In addition, learning proper microscopy techniques is useful for many fields, including biology, chemistry, physics, geology, and many more. **We hope you will be challenged to think like a scientist and that you enjoy this lab module.**

**Learning goals.** To help you better understand how to be a scientist by performing experiments using microscopy techniques.

**Learning outcomes.** By completing this module, we hope that you will finish the course and gain the following outcomes.

<u>Biological research. You will be able to…</u>

- examine how genes/mutations and the environment interact in living organisms
- gain experience in hands on activities that generate useful, biological data
- perform science inquiry by
  - developing a scientific, research hypothesis
  - designing experiments to test a hypothesis
  - collecting data using microscopes, including images that can be used for quantitative measurements.
  - developing models based on their observations, analysis of data, and readings
- communicate science by producing a scientific poster (poster and presentation), containing the introduction, methods, results, and discussion sections
- work effectively in groups to accomplish tasks
- critically read a peer reviewed paper.
- develop science communication skills in the form of lab notebooks, posters and presentations

<u>Microscopy techniques; You will be able to…</u>

- use clinical/research grade microscopes
- learn common sample and slide preparation techniques
- understand how microscopy techniques are performed, their use for answering scientific questions, and their diverse applications across science

- ❖ gain a working knowledge of digital image acquisition, analysis, and data collection using microscopy software (ImageJ)

**Grading summary for this module**
- ❖ Lab Notebook Entries (30%; 5 x 6%)
- ❖ Prelab Quizzes (30% total; 3 x 10%)
- ❖ Lab Worksheet (10%)
- ❖ Final Poster (15%)
- ❖ Poster Presentation (15%)
- ❖ Points will be deducted for groups that do not clean up or participate in lab

**Approximate schedule.** Below are detailed schedule of events and a table summarizing the lab module, including daily activities and assignments.

*Laboratory Day 1:* Introduction to the course project and to microscopes
1) Introduction to the module
2) Learning goals and experiments
   a) Introduction to LabArchives, and electronic notebook entries
   b) Understand the basic use and concepts of light microscopy
   c) Use and identify parts of a compound microscope
      i) Exercise 1: Depth of focus slides on the compound microscope
      ii) Exercise 2: Observe phenotypes under the stereoscope
3) Assignments
   a) ***Individual* laboratory notebook entry 1**.
   b) Read manual for next class.

*Laboratory Day 2:* Introduction to image analysis, quantitation, and ImageJ software
**1) Pre-lab Quiz on materials from Day 1 and Day 2**
2) Learning goals and objectives
   a) Making wet mounts of living material
      i) Cheek cells project (before and after an alcohol based mouthwash)
   b) Digital image acquisition and analysis.
      i) Measurements of cheek cells using ImageJ
3) Assignments
   **a) *Individual* laboratory notebook entry 2 on cheek cells due Friday by 12:00pm or 5:00pm**
   **b)** Read article (Mitchell et al., 2007) by next class

*Laboratory Day 3:* Drunken Worms, Part 1
**1) Pre-lab Quiz on materials from reading (primary article)**
2) Learning goals and objectives
   a) Exercises on scientific inquiry
   b) Measure behavior of wild type animals on control and alcohol
   c) Prepare experiments for Thursday, if necessary
3) Assignments (NONE)

a) ***Individual*** **laboratory notebook entry 3, due Wednesday by 12:00pm or 5:00pm (focus is on Introduction and Methods)**

*Laboratory Day 4:* Drunken Worms, Part 2

**1) Pre-lab Quiz on materials from Days 3 and 4**
2) Learning goals and objectives
   a) Introduction to mutations
   b) Perform experiments on mutants
3) Discussion of format for poster presentation
4) Plan and Perform your experiment
5) Assignments
   **a) *Individual* laboratory notebook entry 4, with discussion questions, due by 12:00pm or 5:00pm on Friday**
   **b) Submit Science Inquiry Lab Worksheet on your project, due at the end of class**

*Laboratory Day 5:* Your experiment

1) Learning goals and objectives
   a) Perform your experiments
   b) Create figures to properly display images for your poster
2) Plan and work on your poster
3) Poster due before next class
4) Assignments
   **a) Submit a *GROUP* laboratory notebook entry 5, due Wednesday by 12:00pm or 5:00pm**

*Laboratory Day 6:* Drunken Worms, Part 4: group poster presentations

1) Learning goals and objectives
   a) Final group planning for poster (First 2 hours of class)
2) Assignments
   **a) 10am or 3pm: Poster presentations (10-12 minutes/group)**
   **b) Posters (in ppt) are due at the time presentations start (10am or 3pm).**

## ii. Introduction to alcohol research and science inquiry

According to the 2015 national survey, 15.1 million adults ages 18 and older (6.2 percent of this age group) had an alcohol use disorder and an estimated 88,000 people die annually from alcohol-related causes (National Institutes of Alcohol Abuse and Alcoholism – NIAAA). However, alcohol consumption is often associated with gatherings with family and friends, celebrations and parties, and increased quality of life. Furthermore, alcohol also contributes to 5.9% of global deaths. Thus, greater awareness of alcohol and its effects on the body is an important preventative measure.

National Institute on Alcohol Abuse and Alcoholism

NIH…Turning Discovery Into Health®

Image from, "Your health! The benefits of social drinking." University of Oxford News

In this module, you will be performing a small project on the effects of alcohol on animals. We will see how ethanol affects body movement and learning. Using techniques learned, you will then perform your own research experiments. This will allow you to follow the process of scientific inquiry, just like a scientist doing research. This includes developing a hypothesis, planning experiments, collecting and analyzing data, and presenting your results.

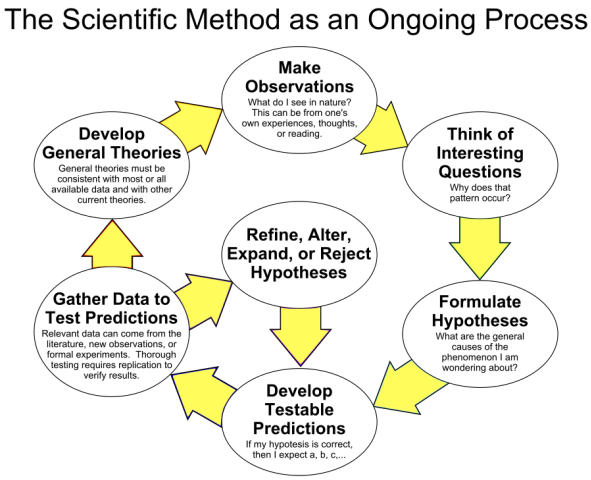



Image is from Wikimedia Commons

## iii. Introduction to Microscopy

Microscopy is the use of microscopes to view objects that cannot normally be seen by the unaided eye. The good, unaided eye can separate objects that are about 0.10 – 0.15 millimeters (mm) apart. This means that two dots closer than 0.10 mm will blur into one dot. However, many biological specimens, such as animal cells, are much smaller. For example, a red blood cell is 6-8µm in size ($10^{-6}$ mm). Thus, to see finer details of objects, our unaided eye needs help.

For scientists, microscopes are valuable tools for many reasons. Microscopes allow scientists to examine specimens in more detail, whether the specimen is a biological sample, a plant, or a crystal structure. Furthermore, microscopes (and cameras) allow us to take pictures of our specimens, make quantitative measurements, and properly document our findings. We can communicate and share these findings with collaborators or other scientists, advancing scientific goals.

### Principles of Light Microscopy

Microscopes take advantage of basic properties of light to magnify objects. Light is energy in the form of waves and particles. Light waves, from light bulbs for example, can hit an object that we see. Light waves will interact with the material in objects, particularly electrons. At this point, as shown in the box insert, light can either be **absorbed** (light is converted to heat), **reflected** (change in angle of propagation of wave to the direction from which it originated), or **transmitted** (passes through). Transmitted light can also be **refracted** (change in the angle of propagation of a wave in the same direction) based on the material the light passes through. Most objects will do a combination. If a light wave is reflected, that light wave then has the opportunity to reach our eye. At that point, we would "perceive" the object. For example, in a pitch-black room, we know there are objects around but we may not perceive them or their location. When the light is turned on, light waves can then interact with object through the processes just described. For purposes of microscopy, light waves (coming from the light bulb in the microscope) will interact with our specimen, and the amount of light absorbed, reflected, refracted, or transmitted can be perceived by our eye or by a camera.

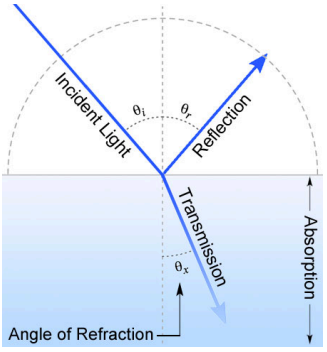


Image from http://performativedesign.com

### Objective lenses

Objective lens, like those found in microscopes, take advantage of light refraction. To magnify objects, we use lenses (objective lenses). Most lenses are made of glass, and light waves coming from specimens will generally be refracted (Figure 1A). A **CONVEX** lens—primarily ones used in laboratory microscopes – will focus light. A **CONCAVE** lens—used mainly in telescopes – will disperse light. Convex lenses have properties – such as diameter, shape, and composition – that will determine the amount an object gets magnified (Figure 1B).

As mentioned, a convex lens will converge light to a point. This point is known as the **focal point** (F) of the converging lens (Figure 1C). There is a focal point in the front and back of the lens. The front of the lens is considered the side where the object is located. The

distance between the center or the lens (vertical axis) and the focal point is the focal length (f). Since F is where a lens focuses light, the values for F and *f* of lenses will determine the ability of lenses to magnify objects.

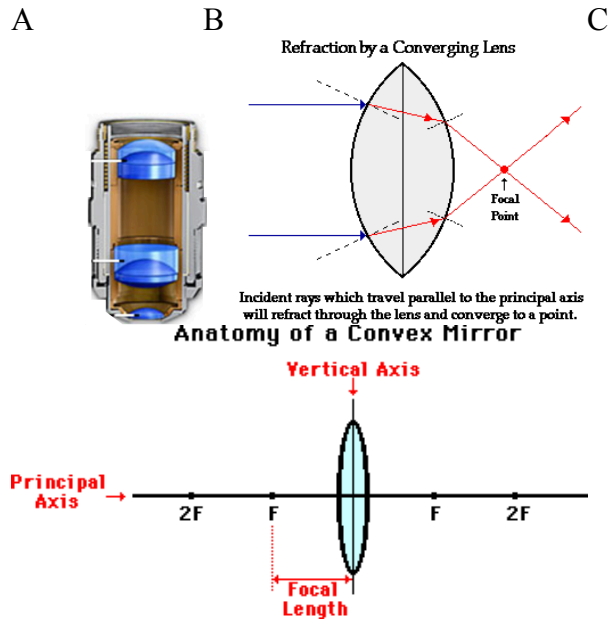



**Figure 1**. A) Microscope objective lens, with differently shaped glass lenses shown in blue. B) Convex (or converging) lenses refract light to a point on the other side of the lens, called the focal point. C) The basic anatomy of a lens, including the focal point (F) and focal length (*f*). Figures from [1].

## Image Formation

Images are representations of objects formed by lenses. The lens in your eye, for example, takes the light coming from an object (button in Figure 2) and focuses an image to the back of your eye (retina). Microscope lenses do the same, but rely on a collection of lenses to manipulate light waves. Because there are multiple lenses, these microscopes are called **compound microscopes**.

In this microscopy module, we will explore the basic principles and applications of microscopes. There are complex physics and math principles involved with light microscopy, but they are detailed and beyond the scope of this class. For those interested in advanced microscopy, please see [3].

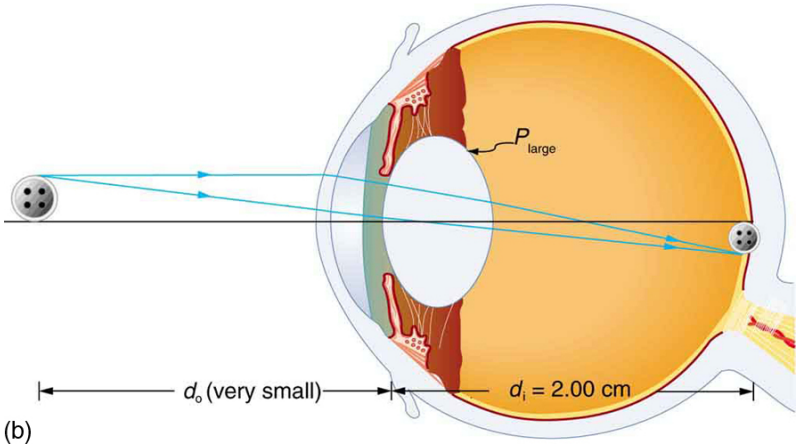



**Figure 2**. Image formation by light entering the lens of the eye. From [2].

Microscopes

Depending on your application, you will need to choose which type of microscope and which microscope technique you would use to answer your question.

You will use two types of microscopes in this lab.
1) Compound microscopes
2) Stereomicroscopes, or dissecting microscopes

Types:

- *Compound microscope*: uses high magnification objectives to reach the limits of **resolution**, which is dictated by the wavelength of light, at approximately 0.2um
- *Stereoscope* (dissecting scope): uses lower magnification light but has large **working distance** between the objective and sample, allowing manipulation of sample while using objectives
- *Electron microscope*: uses electron beams resulting in a smaller wavelength than light waves, allowing electron microscopes to have a much higher resolution than optical scopes

Techniques:

- *Bright field*: samples are illuminated via transmitted white light from below or above the sample
- *Dark field*: aligned light source minimizes the quantity of directly transmitted (unscattered) light entering an image plane
- *Phase contrast*: shows differences in refractive index as difference in contrast
- *Differential interference contrast*: allows detection in differences of optical density
- *Fluorescence*: specimens are illuminated with high energy light which causes the emission of a different, lower frequency light

Other considerations

**Resolution**. Resolution is the ability to see two objects as distinct (Figure 3). Resolution is dictated by the wavelength of light and the properties of the objective you use. Basic compound microscopes can resolve two spots to approximately 0.2um.

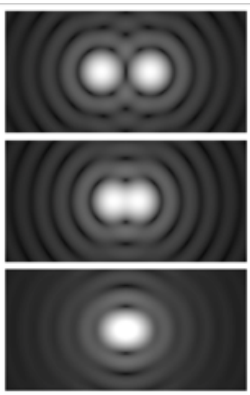


**Figure 3.** *Top*, two white spots are far enough apart that they appear as two independent spots. *Bottom,* as the spots get closer (or your objective resolution decreases), the spots may appear as one.

**Depth of field.** Normally, we think of resolution only on the lateral axis of the specimen (left to right). However, there is also resolution along the longitudinal axis (top to bottom). Because of this, objects may only be in focus along a small plane of view, and objects above or below the main plane will be blurry. Similar to lateral resolution, depth of field resolution will depend on the properties of the objective.

**Total magnification.** Total magnification is determined by multiplying the magnification of the objective lens times the magnifying power of the ocular lens (eyepiece). For example, if you are using an objective lens that has 10x magnification, and an ocular lens that has 5x magnification, your total magnification is 50x.

### iv. *Caenorhabditis elegans* (*C. elegans*): A model organism to study biological questions

The nematode *C. elegans* is a model organism used to study myriad biological processes ranging from cell biology to behavior. *What is a model organism?* Model organisms are animals we use that can help us better understand basic functions of biology, such as gene function, cell function, or intracellular networks, without having to perform research on humans. *C. elegans* share some qualities that make them "comparable" to human systems. They are multicellular organisms (approximately 1000 cells) and have tissues like other mammals, such as nervous, muscle, and digestive systems. They do not have a heart or cardiovascular system, but do have a "circulatory" system to deliver chemicals. They have many of the same chemical messengers such as hormones and neurotransmitters. Furthermore, many of the genes found in humans are found in worms, with high genetic conservation. Thus, we can use the model organism *C. elegans* to study the function of genes and cells, which may be conserved humans.

**Figure 5**. *C. elegans.* Image is from [4].

There are some differences as well. They have two sexes, hermaphrodite and males. Hermaphrodites reproduce clonally; this actually makes worms an ideal tool to study genetics, because genotype and phenotype are tractable from generation to generation. One hermaphrodite can produce 200 eggs or more that are all the same genetically. They are found in soil and feed off bacteria in the soil. Wild type (N2) animals are only approximately 1mm in length and move in a well-defined, sinusoidal pattern while foraging for food and exploring their surroundings. Because of their stereotypic movement, we can more easily examine what factors (chemicals or genetics) alter "normal" behavior. Thus, in this module, we can use *C. elegans* to better understand how particular genes or factors alter behavior.

Did you know?

- Six *C. elegans* researchers have been award the Nobel Prize for their work, ranging from animal development to cell death mechanisms to the use of green fluorescent protein!
- In 1998, *C. elegans* was the first multi-cellular eukaryotic organism to have their entire genome sequenced!
- About 35% of *C. elegans* genes are closely related to genes found in humans.
- About 50-60% of the human genes known to be associated with "diseases" have a homologue in the worm.

# Day 1

## i. Day 1 BACKGROUND

First, you will familiarize yourself with a **compound microscope** (Figure 4A). These microscopes are used to view specimen on glass slides, with high magnification objectives (4x – 100x range). In exercise 1, you will learn how to care for a microscope, the parts of a microscope, and techniques to improve image quality. You will then use the microscope to practice your technique and to better understand resolution.

In exercise 2, you will familiarize yourself with a **stereoscope** (Figure 4B), which is also called a dissecting microscope. Stereoscopes utilize lower magnification lenses, but have more space between your specimen and objective. This space is called working space, which is more flexible for some applications because the experimenter can dissect, manipulate, or move the specimen.

Care of a Microscope

Microscopes are expensive equipment! Be careful at ALL times when handling a microscope or its parts. Here are some tips to help you:

1. When working with objectives, start with the lowest magnification, focus on your specimen, and gradually move to the next magnification. (If the objective hits your sample while you are turning the revolving nosepiece, that is NOT good.
2. Do not force knobs to turn or parts to move. Most movable parts of a microscope move easily.
3. If you do need to move the microscope, use two hands and make sure to hold a microscope firmly by the stand, only. For example, do not grab it by the eyepiece holder.

When you are done with your microscope:

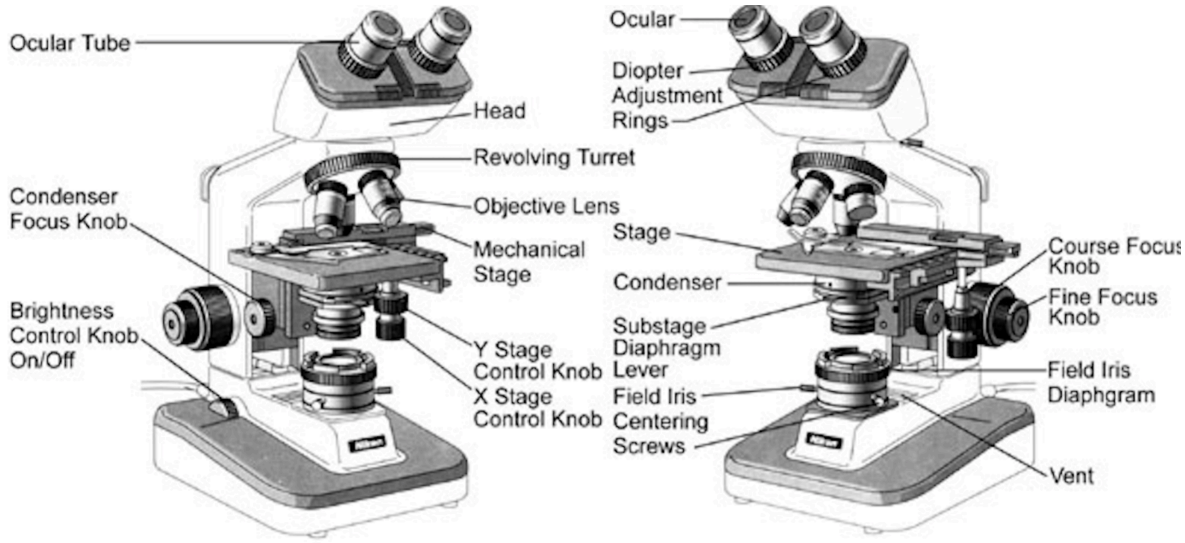
1. Turn the power off (knob on the left base of the microscope).
2. Cover the microscope with the protecting dust jacket.

Parts of a Microscope (refer to Figure 4)

- Ocular: eyepieces through which the sample is viewed; the oculars have a level of magnification of 10x; may have adjustable **diopters** to adjust for differences in eye prescription
- Objective: magnification of each is printed on casing, ranges from 4x (low power) to 100x (high power)
- Revolving turret: rotating wheel that allows the user to switch between objectives
- Focus knobs: course and fine focus knobs shift the distance of the sample from the eyepiece to move the sample to the focal point
- Stage: surface to mount sample slide, notice the aperture (opening) which allows light to pass through to the sample
- Condenser: an adjustable set of lenses that focuses the light, higher magnifications generally require more light; located on the condenser is the **aperture diaphragm** which is adjusted to correct for contrast in Kohler illumination

- Field lens diaphragm: adjusted to correct the aperture of the light source for Kohler illumination
- Light source (illuminator) can be diascopic (lit from the bottom) or episcopic (lit from the top)

A




B

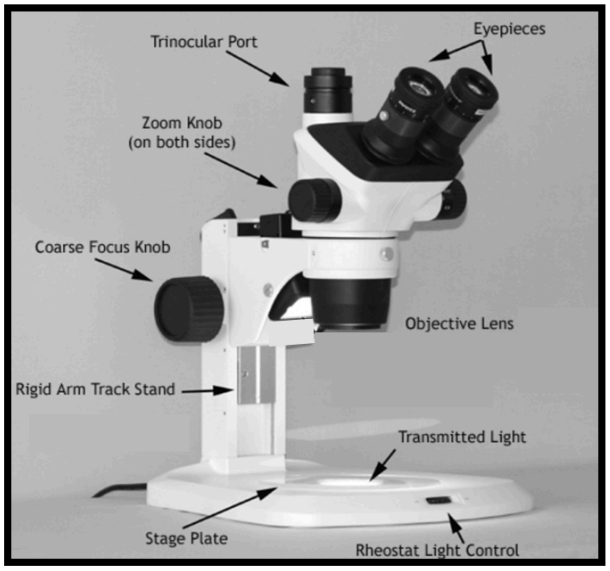



**Figure 4.** Parts of a microscope. A) Compound Microscope (Image from Nikon) B) Stereomicroscope (image from Microscope World).

*Köhler* Illumination

Simply put, *Köhler* Illumination is a technique to optimize the lighting conditions of the specimen under view (by the eye or camera). This is a step-by-step technique a microscopist performs before sitting starting his or her experiments. It helps the experimenter attain the best image of the specimen by affecting illumination in two ways: 1) evenly distributes illumination throughout the specimen and 2) balances resolution and contrast. *Köhler* Illumination adjusts two diaphragms (similar to shutters), to control light. One adjustment is the **field diaphragm**, which controls the area of the specimen that is illuminated. This should only be the field of view (view field) through the eyepieces. The second adjustment is the **aperture diaphragm**, which controls a compromise between resolution and contrast. With the aperture diaphragm, wide-open maximum resolution is achieved. With the aperture diaphragm closed all the way, the specimen will have maximum contrast, but at the expense of low resolution. Incorrect adjustment of either diaphragm will limit the resolution – you will not be able to see the fine details in the specimen as well as possible.

A frequent misconception is that opening or closing one or both of the diaphragms can control the brightness of the image. False! Adjusting the illuminator brightness control affects the brightness. The above procedures become "reflexes" for anyone who uses a microscope routinely. Following these steps before every experiment will you get the maximum performance from any microscope. See your worksheet for a step-by-step procedure on how to set up your microscope for *Köhler* Illumination.

## ii. Day 1 PROCEDURES

1. First, read through the parts of the microscope, and identify all the parts.

2. Use the worksheet to set up your microscope for *Köhler* Illumination. It is expected that all images submitted for future assignments will use *Köhler* illumination techniques.

EACH PERSON SHOULD ANSWER QUESTIONS IN YOUR LAB ARCHIVES (LA) NOTEBOOOK. **You will answer questions directly on this day's entries, but must identify your work by changing the color of your text.**

3. Exercise 1: using a compound microscope
   1. Examine your slide with colored film with your eye. Can you determine the order of the colored lens paper with your naked eye? Answer on Lab Archives by recording your observations.
   2. Using the compound microscope, move the objective to the lowest magnification (4x objective).
   3. Place the slide of colored film under your microscope objective. **We always start with the lowest objective.** Center the crossed films over the aperture. While looking at the slide with the 4x objective, adjust the focus until the crossed colors come into focus. Can you determine the order of the colors using the 4x objective? Answer on Lab Archives by recording your observations.
   4. Now, determine the order of colors by using different objectives (move up gradually) to determine which is best suited for this task. Which objective was best for determining the order? Answer on Lab Archives by recording your observations.

4. Exercise 2: using a stereoscope
   1. Using the stereoscope, examine your wild type (N2) plate of *C. elegans.*
   2. To properly view objects under a stereoscope, you must also use the correct lighting and magnification conditions. For worms, which are transparent, it is best to use the matted reflector, not the mirror reflector. Also, start with the lowest magnification and gradually zoom in.
   3. Examine the mutant plates at each station for a few minutes and enter your **observations** in your lab notebook (LA). Observation is one of the first and most important steps of science.
      - Identify 3 adjectives that describe each of the mutants.
      - For each mutant, try to postulate a biological reason that could lead to the phenotype(s) you observed.

5. Exercise 3: Practice picking worms (see "Worm picking 101" below) (optional)
   1. Sterilize your pick by passing the metal wire through your flame. **Make sure the ethanol burner/flame is positioned to not burn your hands or any wires/cords.**
   2. Then gently use the metal pick to attempt to move 3-5 animals to a new plate. Label the bottom of your plate with your initials, genotype, and date.
      The **goal** here is to successfully move only adult animals without killing the animals and without puncturing the agar. Using a stereoscope requires good depth perception

and manipulation of tools only through visualization through lenses. This is good practice for any microscopist!

## Day 2

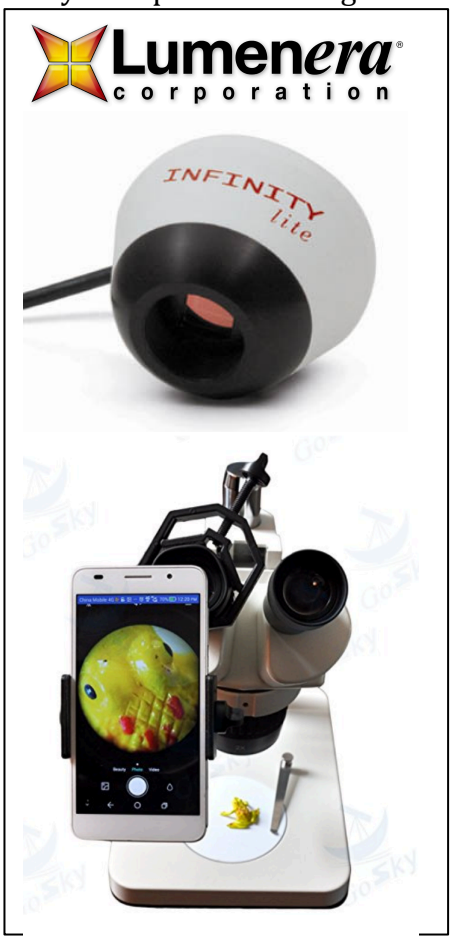
### i. Day 2 BACKGROUND

Introduction to Image Acquisition and Analysis

Documentation is important in science, because it allows the experimenters to record their findings, quantitate data, and communicate the information to fellow scientists. It is also part of a good laboratory notebook. For this, you not only need your microscope but also a microscope camera, camera software, and a computer to run the software. Furthermore, advanced image analysis may require other software (but some camera companies include analysis packages in their software).

Image acquisition with Infinity Analyze

Today, many microscopes are equipped with the ability to capture the image of the specimen. **Image acquisition is the process of taking the image of the specimen you see with your oculars, and digitally storing it (in a file)**. Usually, this is done using a camera mounted in the optical path of the microscope. One camera you will use (INFINITY lite, Lumenera Corp., top figure on the right) is a color digital camera but many cameras are also black and white (called monochrome). The cameras have a chip in it that collects photons of light waves (similar to the retinal cells in your eye), and converts the photons to an intensity value. Color cameras will be able to collect RGB, mean red, blue, and green wavelengths. The resulting computer image is a grid representation of the intensities (pixels). The lite camera has a 1440x1080 pixel (1.4MP) resolution. *What resolution does your smart phone camera have?*




Speaking of smart phone cameras, we will also be using our phones to capture some images (mostly because it is easier than switching cameras between microscopes and to allow for more students to use microscopes at once). For this, we have an adapter (Gosky) to mount our phones onto the eyepiece. The instructor or TA can help you with this. With many phones nowadays, the quality of the image might be even better than some cameras!

Cameras must be run by software on a computer (or your phone). For this lab, the software is called *Infinity Capture*. *Infinity Capture* has a live display setting (live in Image menu), a capture setting, and a video recording setting. All of these can be saved for analysis in other platforms (for example, as a jpeg or mp4).

Image analysis with ImageJ

ImageJ is a public, image-processing program that is made available by the National Institutes of Health (NIH). It can display, edit, analyze, process, save, and print many types of images. For this lab, you will use it to analyze the images you take. In day 2, you will analyze your own cheek cells. In days 3-5, you will analyze the videos for your experiments on genes regulating ethanol sensitivity. Because of the utility and cost (free) of ImageJ, it is a powerful tool to know and use. See the tutorials for specific instructions.

## ii. Day 2 PROCEDURES

Exercise 1: Examination and quantification of epithelial cell size

For this experiment, you will compare the size of your cheeks cells before and after rinsing with mouthwash. This exercise will provide you experience with

- preparing microscope slides
- use compound microscopes, cameras, and image acquisition software to collect images
- use image analysis software to analyze data
- perform an experiment that has one testable variable (mouthwash)
- communicate your results in a manuscript format


Figure 8. Listerine and label

*Collecting cheek cells*

**Note: don't all try to complete the procedure at the same time! There are two compound scopes per table, so only two people can prepare their cells at a time. Stagger yourselves so you don't ruin your samples. Some students work on Exercise 2 or the ImageJ tutorial while you wait.*

1. Set up Kohler illumination.
2. Set up camera.
3. Collect cheek cells by scraping the inside of your mouth with the flat side of the toothpick.
4. Rub the toothpick on the center of the microscope slide
5. Using another toothpick, dip one end into the methylene blue solution (collect a small drop); add dye to the area where you placed your cells on the microscope slide, and mix.
6. Cover with coverslip
7. Seal the slide with nail polish by applying the polish over the sides of the coverslip
8. Use the compound microscope to visualize your cells. To take pictures, use the camera phone mount. If you do not have a camera phone, please see if your classmate will take pictures with his/her phone. It is easiest to keep one phone on the mount once you have it set up.

   a. Send yourself the pictures of your cells, and post ONE example in your lab archives notebook.
   b. Document in your lab notebook (LA) what your cheek cells look like.
9. Capture images of 5 cheek cells.
   a. In your notes, make sure you record What objective you used?
10. Rinse your mouth with mouthwash (Listerine, 27% alcohol)
   a. Swoosh the listerine solution in your mouth for two minutes
11. Repeat steps 1-7.
   a. Use the **same objective you used for your control cheek cells**.
   b. Measure 5 cells of your mouthwash-rinsed cheek cells.
   c. You will use your image files for analysis later
12. Using the same objective you used for your cheek cells, take a picture of your micrometer (in the purple box). This will be necessary for your ImageJ analysis of cheek cell sizes.

*Image acquisition and analysis using your cell phone*

1. Mount the Gosky camera adaptor to the eyepiece.
2. Place your phone into the mount, with the camera lens in the center of your microscope eyepiece. This may take some adjusting, as it need to be nearly perfectly aligned.
3. DO NOT change to zoom of your camera. It should be at 1x zoom as you will need to compare images of the same size.
4. Take a picture of your sample (follow directions above for sample preparation).
5. Take a picture of the **scale bar**.
6. Transfer your pictures from your phone to your computer.
7. Follow the ImageJ protocol to measure the size of your cheek cells.

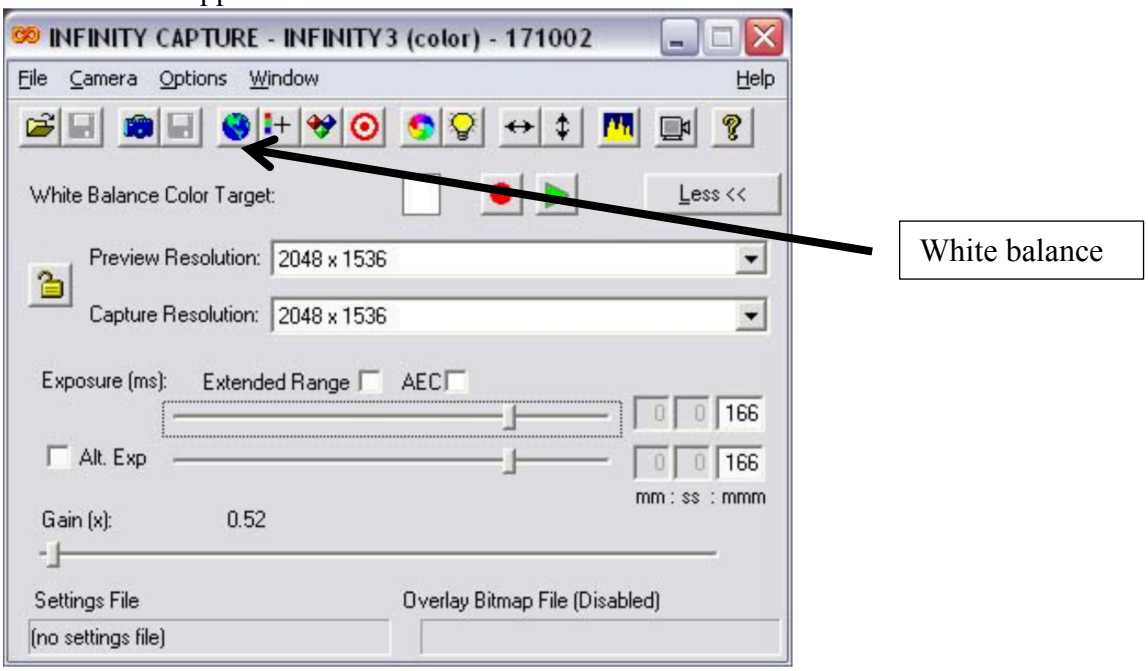
*Image acquisition and analysis using Infinity Lite*

Image Acquisition using *Infinity Capture*
Below, a brief tutorial of the Infinity Analyze software is provided. If necessary, however, use the following link to find tutorials on using the Infinity Analyze software.
(http://www.lumenera.com/support/microscopy/training.php)

Briefly…

**Infinity Capture (for taking pictures)**

1. Open Infinity Capture (shortcut located on your desktop). Once opened the following screen should appear:

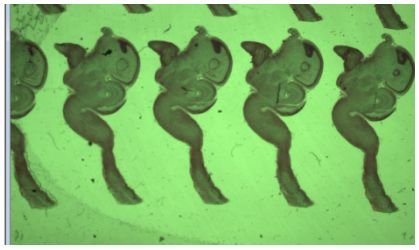


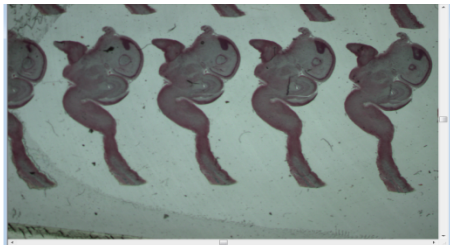

2. In order to view your slide on the microscope, ensure that the camera shutter knob (located just behind your eyepieces) has been pulled out on your microscope. This opens the light path to the camera.
3. Make sure to focus your image.
4. Things you can try to change to get quality images:
    a. **Exposure** – this is the amount of time you allow the camera to take in light, or photons. Exposure can be manually controlled using the sliders, but I suggest you use auto exposure feature.
    b. **White balance** –basically ensures that objects which naturally appear white are shown as white; once the color palette is adjusted to this standard all other colors should appear in their natural form allowing for the best visualization of details
        i. For the white balance function in Infinity Capture by going to camera >white balance to find two options.
            1. One shot global (same as selecting the earth icon on the main screen). This method balances light over the entire field. It is

suggested that you more to a clear area of the slide. (arrow in figure above)

2. One shot spot. This allows you to pick your area to use as a reference point. A crosshair will appear, which will allow you to choose your own area, which is a 32x32 pixels spot.

ii. White balance before and after:





c. **Gain** – Increasing gain allows you to increase the sensitivity of the camera without increasing exposure. The slider is at the bottom of the main options menu. However, the image quality may decrease as the gain is adjusted upward because the noise of the image will also be amplified. More is not always better!

5. Once your image is white balanced and focused, you can take a picture in Infinity Capture. Press on the blue cameral button. This will bring up a new window.
6. In order to save your image you must select it, go to File>Save as. The image will be saved as a TIFF(*tif) file by default; however the image can also be saved as a JPEG depending on computer compatibility. The file will be around 1000Kb. **REMEMBER, save all information to a desktop folder, but immediately move them to a flash drive (attached to computer or your own). Any data found on the computer will be immediately deleted and may result in loss of your work. Any files cluttering the desktop will result in the loss of points for your group.**

**How to save your files to any flashdrive.**

1. Open up the folder where your file is present.
2. Right click on the folder located on the desktop and select 'Windows Explorer'. Find the folder that is your Flashdrive's name. You can highlight your files and drag them into the Flashdrive folder.
3. Save your file as a TIFF file to a desktop folder. It is good practice to save your files in a logical order (e.g. [LASTNAME]_cheek_ctrl_01).
   ***NOTE*** **All files must be moved onto a flash drive. These computers have very little memory. Any files found on the computer will lead to a deduction of points from you or your group (at the discretion of the instructor).**

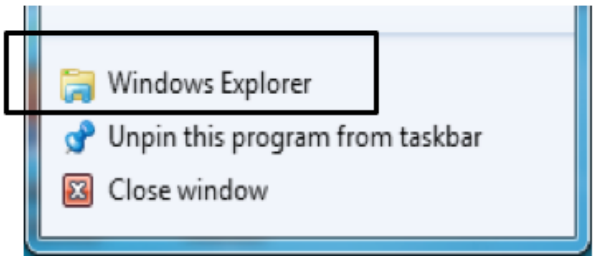



Image analysis using ImageJ

1. Follow the ImageJ tutorial to measure the sizes of cheek cells. Consider and answer the following questions in your lab archives notebook entry.
   - What is the average size before wash?
   - Do you predict a rinse would alter the size of the cells? Explain your answer in your discussion.
   - Does the rinse change the size of the cheek cells you measured – point out specifically how your data support your hypothesis/prediction?

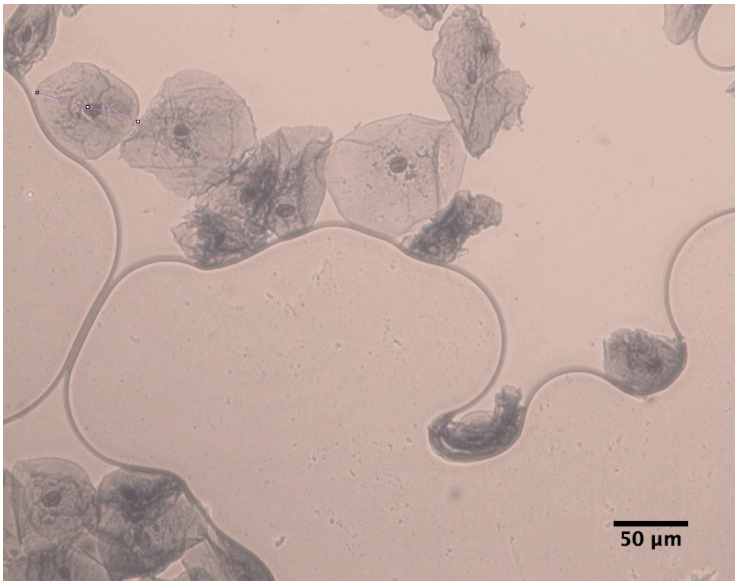


**Figure 7.** Example image of cheek cells stained with methylene blue. Image was acquired with Scion VisiCapture, and analyzed using ImageJ.

Exercise 2: Practice moving animals

1. As in last class, you used a stereoscope and a sterile pick to practice moving adult wild type animals to a new plate without puncturing the agar.
2. Practice until you can successfully move 5 worms from one plate to the next, without killing worms. You will need to do this for your experiment next class.
3. Have TA or instructor check your plates.

## Day 3

### i. Day 3 BACKGROUND

**Microscopy Part II: Drunken Worms Project**

Last week, you were introduced to *C. elegans* as a model organism for research, and got familiar with microscopes and microscopy software. Furthermore, you performed an experiment testing one-variable (mouthwash) and how it affects the size of your cheek cells. Using these inquiry and technical skills this week, you will be starting your analysis of the effects of alcohol on animal behavior, specifically movement and learning. **Alcohol (ethyl alcohol or ethanol) has varied effects on humans,** including states of euphoria as well as states of depression. It can also impair cognitive function, motor coordination and judgment. Thus, alcohol is thought to affect the functions of **neurons and muscles**, or both, that control behavior. You will be examining how alcohol affects animals by testing two variables: alcohol concentration and host genotype. First, you will test whether different concentrations of ethanol (**variable one**) affect the movement and learning of normal (wild type) worms. Alcohol is thought to work on the nervous system, or the brain, to alter the communication between neurons (Figure 9). The chemical ethanol itself can alter membrane properties of neurons, or directly bind to receptors for several types of neurotransmitters, including acetylcholine, serotonin, glutamate and GABA. However, many of the factors, including the genes in neurons that are important for alcohol's functions, are still being studied. Thus, you will also examine what happens when we introduce another variable: host genotype (**variable two**). For this, you will repeat your experiments using worms containing a mutation in one known regulator of alcohol function, a potassium channel known as *slo-1*.

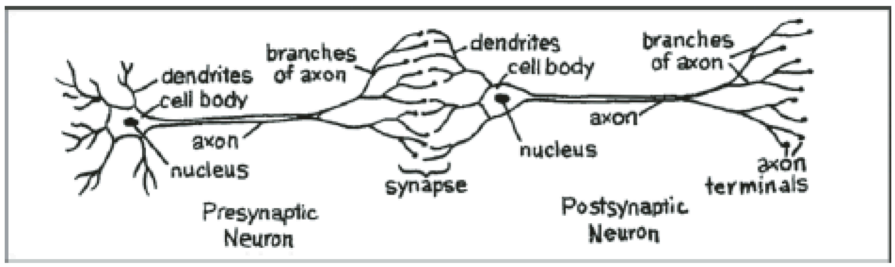



**Figure 9. Diagram of neuronal connections in the nervous system.** Neurons communicate with each other at contact points called synapses. Synapses are points of communication between two cells, such as two neurons diagrammed on the top. Image is from Wiki Images.

Alcohol can have euphoric or depressive effects, including effects on neuronal function, movement, and feeding behavior. While the exact effects of ethyl alcohol on the nervous system are complex, some of the physiology at synapses are well known (Figure 10). For example, one function of ethyl alcohol is to bind GABA receptors of neurons in the brain, which are chloride channels. They enhance the effect of the receptor, allowing more chloride to come into the cell. Chloride is a negatively charged ion, and increased influx of chloride into neurons makes neurons more negatively charged. The outcome is that this depresses the activity of neurons, leading to the depressant effects of alcohol.

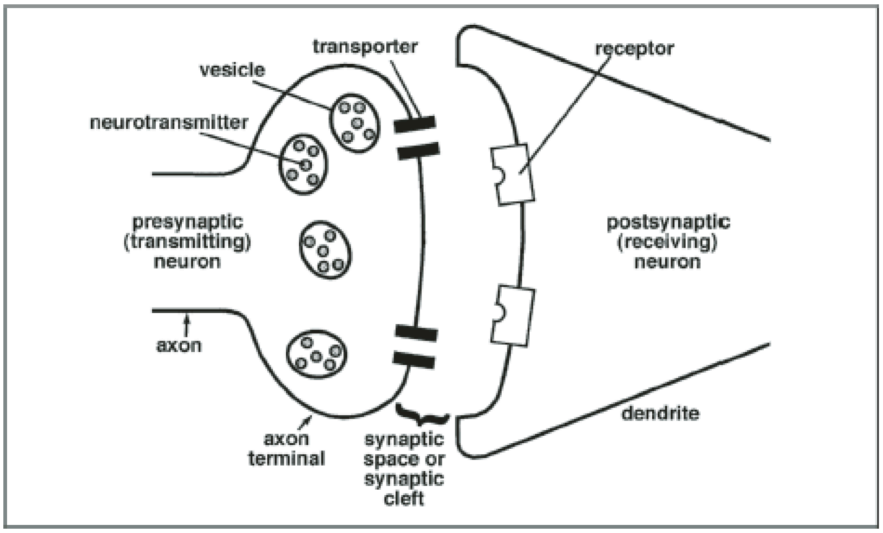



**Figure 10. Diagram of synapses in the nervous system.** A cartoon of a synapse is shown. Neurotransmitters are released from storage vesicles, or synaptic vesicles, of a presynaptic neuron. After release, neurotransmitters can act on receptors on a postsynaptic neuron and cause a change in signaling of the postsynaptic neuron. Thus, the presynaptic (transmitting) cell has communicated information to the postsynaptic (receiving) cell. The information is often determined by the amount of neurotransmitter released, or the ability to sense the neurotransmitter. Many drugs act at the level of synapses and alter communication between neurons. Images are from the National Institutes of Health (NIH). Image is from Wiki Images.

To better explore how ethanol affects movement and learning, you will quantify changes in the behavior of worms. In one study, you will examine a movement of the body wall muscles (kind of like skeletal muscle of your body) in response to different doses and exposure times to ethanol. Specifically, you will examine a behavior called thrashing (swimming) to look at body movement (Figure 11). You will be treating worms with different amounts of ethanol, and examining how movement changes at these concentrations. We expect to see results similar to those described in the paper you read by Mitchell et al. (2007). To do your experiment, you will perform the behavioral experiments, observed the worms by eye or by acquiring videos. Then, you will make graphs to display the results of your experiment. We will not be performing statistics in this module.

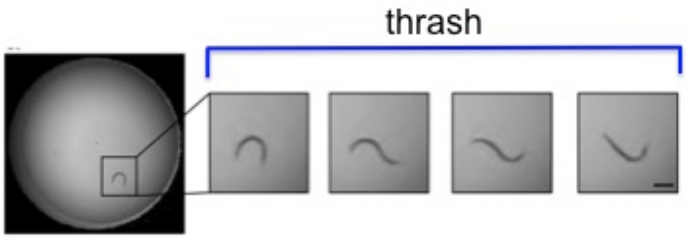



**Figure 11. Measuring movement in worms during swimming behaviors.** Worms thrash in water, which can be analyzed in videos. To measure thrashing, analyze worms moving from one side, crossing a midline to the other side, and back (one thrash).

In another experiment, you will examine the effects of ethanol on a learned response. The learned response we will examine is starvation-induced NaCl aversion. This is an associative learning paradigm. Specifically, worms are put on a plate without a food source (without bacteria, or starved) and with or without NaCl. Worms put on plates without food and with NaCl (starved + NaCl) associated NaCl with starvation, which they did not like (Saeki, Yamamoto, & Iino, 2001). Later, they presented worms with a choice to move towards a spot of NaCl or a control spot, and more moved towards the control (Figure 12). Worms that were not pre-exposed to NaCl (naïve worms) either spread out equally or even moved more towards the NaCl.

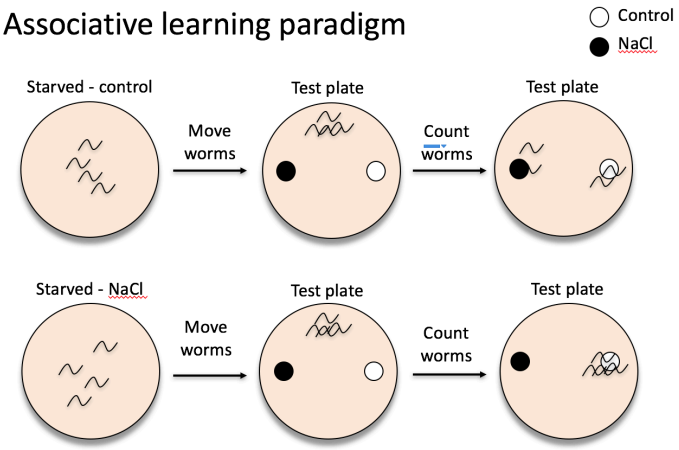



**Figure 12. Associative learning paradigm in worms.** In learning paradigms, you first condition animals by putting worms on plates with and without the associative cue (NaCl) in an unfavorable condition (starvation). You then moved the worms to a test plate that allows them to choose between the cue and another non-associated cue (in most cases a control solution). After some time, worms will move away from the cue if they have been pre-conditioned to associate NaCl with starvation.

Using this associative learning paradigm, we will examine how pretreatment of ethanol affects the ability of animals to make the association. As alcohol consumption is known to affect cognitive function, experiments like these will help us better understand the biology of alcohol and learning.

## ii. Day 3 PROCEDURES

Exercise 1: Quiz and Discuss paper

1. The quiz will partly be on the paper by Mitchell et al. (2007).
2. Discussion of alcohol

Exercise 2 &3: Wild type learning and movement after ethanol treatment.

General procedure for washing worms

1. Take one plate of gravid worms and gently wash the worms off the plate using the 1mL pipette tip (with a p1000 pipetman). Pipette up **1ml of M9 wash buffer** and add it to the plate of worms. Wash at least 3 times by pipetting up and down (some worms get stuck on the plastic pipette, but that is ok). **Do be careful to not aspirate the fluid into the pipetman!** Then, put the M9 (with the worms) into a 1.5ml eppendorf tube.
2. Allow the animals to settle to the bottom, for approximately 5 minutes.
3. Once most animals have settled, carefully remove the supernatant with a p1000 pipetman. You can leave about 200μL of solution.
4. Add 1mL of fresh M9.
5. Remove all but 200μL of solution, approximately.

Exercise 2: Wild type associative learning after ethanol treatment

1. After washing your plate of worms, you will split them up into no-EtOH and (+)EtOH treated conditions (2 plates, 2 tubes) Carefully remove the supernatant but leave 200μL of worms in each of your your wash tube.
2. Label the tubes (no EtOH) and (+EtOH). If you only have one tube, mix worms in the 200μL and Move 100μL to two different 1.5mL Eppendorf tubes.
3. For the no EtOH tube, add 800μL of M9; For the +EtOH tube, add 770μL of M9 AND 30μL of 95% EtOH (roughly 3%, or 500mM)
4. Let them sit in this solution for 15 minutes. Gently flick the bottom of the tube periodically to mix worms.
5. After 15 minutes, make sure worms have settled to the bottom of the tube. Remove supernatant but leave 200μL. (do this gently by not disrupting the worms at the bottom of the tube)
6. Mix the 200μL and add 100μL of worms from each of the following conditioning plates (which have been prepared for you). You should have two plates each. Look under the microscope and aim for >100 worms (roughly). If you feel there are less, add more volume if you have left over.

    1) Control/$H_2O$ conditioning plate
    2) NaCl conditioning plate

    REMINDER* - prior to adding worms, give the bottom of your tube a flick/tap to mix up the worms, so you don't pipette too little or too many worms to one plate by mistake.

**Thus, these are your final 4 groups**

1. No EtOH + $H_2O$ control
2. No EtOH + NaCl
3. +EtOH + $H_2O$ control
4. +EtOH + NaCl

7. Keep lid open to air dry to solution.
8. After the plates are COMPLETELY dry, close the lids.
9. Wait one hour for the worms to equilibrate to their plate condition.

THIS IS A GOOD TIME TO START YOUR THRASHING EXPERIMENT (Exercise 3)

1. 15minutes prior to your 1 hour, prepare your **Test plates**. For this, you will add a chemical called sodium azide ($NaN_3$) to the control (white) and NaCl spots (black) (see figure). $NaN_3$ will help paralyze the worms at that spot and make counting easier. Carefully add 2µL to these spots. Be careful to not puncture the agar plate. Close lid and let the $NaN_3$ dissolve into the plate.
2. After the conditioning, you will wash worms from each plate. To do this, you will use a p1000 pipetteman to wash on 1000µL of M9 wash buffer. Do this by tilting the plate slightly, and adding the wash buffer to the top of the plate allowing the buffer to fall down the plate and get the worms, as described above. Collect worms in a 1.5mL Eppendorf tube.
3. Allow worms to settle to the bottom (few minutes)
4. **Repeat** wash by CAREFULLY removing the supernatant, leaving about 100-200µL.
5. Add fresh 1000µL M9 wash buffer.
6. Allow worms to settle to the bottom (few minutes)
7. Return to your Eppendorf tubes, and remove most of the solution. Leave about 100µL of worms for each tube.
8. Add 25µL of worms to the drop spot on your scoring plate (see figure). You can pipet from the bottom of the tube
9. After 30 minutes, record the number of worms on each part of the plate:
   1) the control zone
   2) the NaCl zone
10. Use Infinity Capture to take pictures of each zone. See seperate instruction document.
11. Chemotaxis Index. Use your numbers to get a chemotaxis index for each of the 4 groups. For this, perform the following calculation. (ignore the other zones)

$$\textbf{Chemotaxis Index} = \frac{\text{No. of worms on NaCl zone} - \text{No. of worms on control zone}}{\text{Total no. of worms (No. of worms on NaCl zone + No. of worms on control zone)}}$$

12. Record your numbers in your notebook

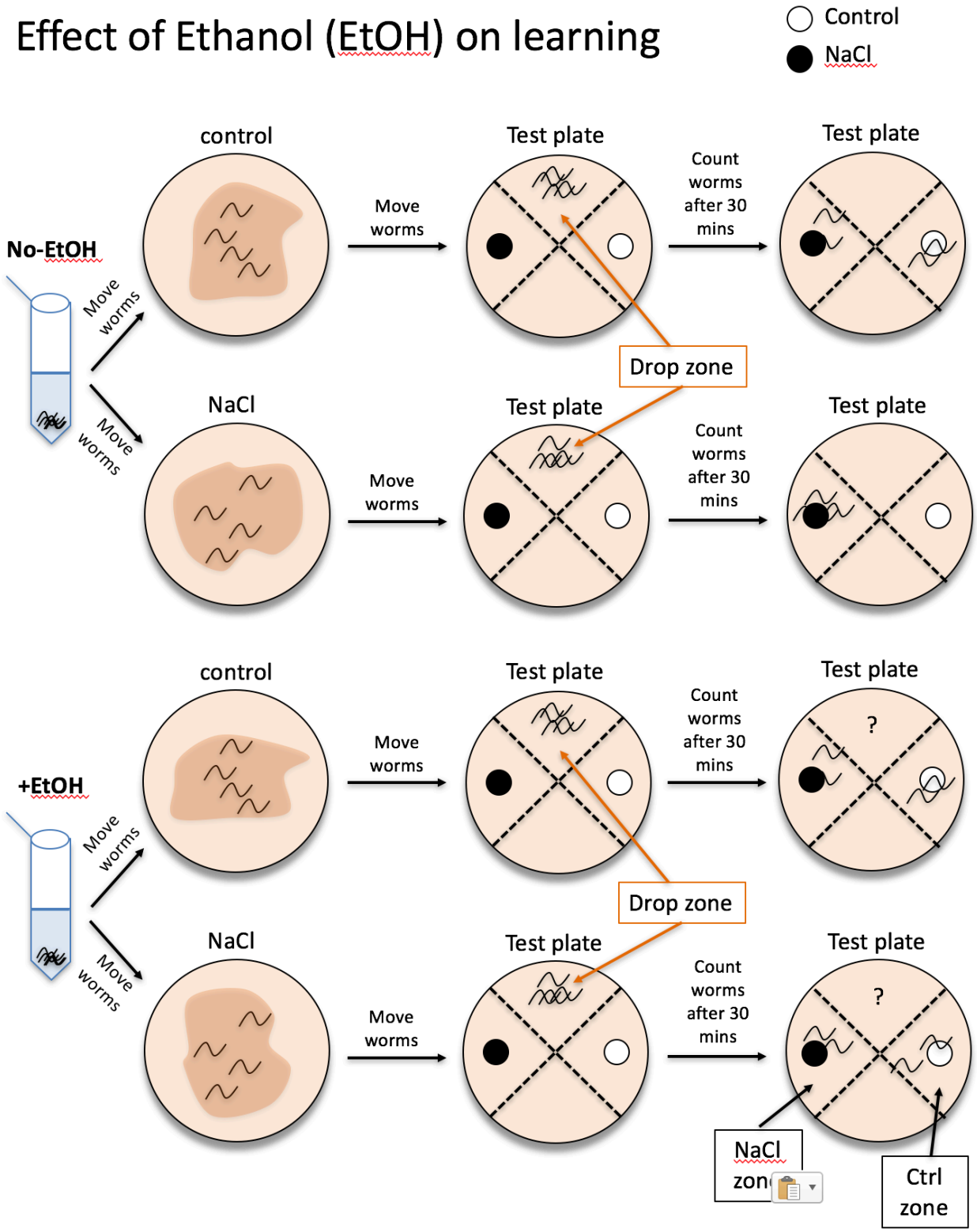

Effect of Ethanol (EtOH) on learning
Control
NaCl
No-EtOH
+EtOH
control
NaCl
Move worms
Test plate
Count worms after 30 mins
Drop zone
?
NaCl zon
Ctrl zone

**Figure 13. Diagram of the procedure for measuring the effects of ethanol on associative learning in worms.**

Graphing your data. After filling out the table in excel, you should be able to graph your data, such that the results are easy for the reader to comprehend (see figure 14). Please ask your TA or instructor for help with data if necessary. Using excel is easiest, see the tutorial.

1) Enter your chemotaxis index (CI) numbers into the cells as shown (Figure 14)
2) Graphing and correctly labeling graphs are important parts of science communication. Below is an example of graphs that you should produce, and have on your lab entries.

A)

| Wild type worms (day 3) | | | | |
|---|---|---|---|---|
| no. worms | No-EtOH Ctrl | No-EtOH NaCl | (+)EtOH Ctrl | (+)EtOH NaCl |
| NaCl zone | 19 | 39 | 34 | 34 |
| Ctrl/water zone | 11 | 7 | 27 | 31 |
| Chemotaxis index | 0.27 | 0.70 | 0.11 | 0.05 |

B)

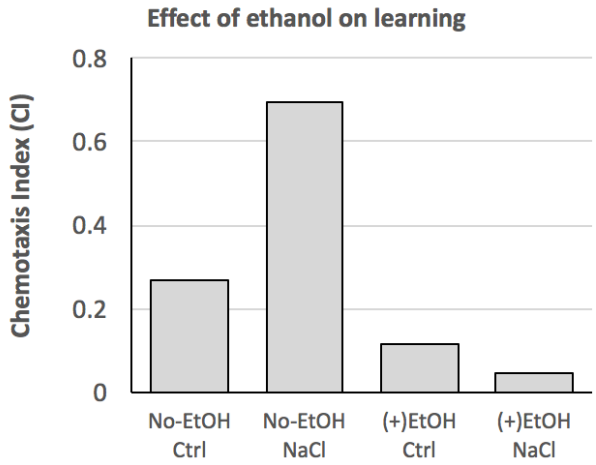



**Figure 14. How to graphically depict data showing the effects of ethanol on learning in *C. elegans*. A)** An image of how an excel entry might look like. **B)** A bar graph of example-*fake* data showing the chemotaxic indices of worms in the four different conditions. CI, chemotaxic index; Ctrl, control

Thrashing assay

1. Follow General Wash Procedure to wash your worms. When you are ready to add your worms for your experiment, mix the worms by flicking the bottom of you tube before pipetting. Mixing worms is important so that you don't pipette up all M9, or too many worms for your experiment.
2. Add worms to the glass slide (with depression, see figure below).

**DEPRESSION SLIDES ARE NOT TO BE THROWN AWAY!**

A depression slide

3. After adding your worms to the depression slide, add the right amount of ethanol to get to your desired concentration to test your worms. **Use the table below; notice that the volume of the stock and worms change to get to some of the desired concentrations.** Not all concentrations are used. For your control, add fresh M9 instead of ethanol (see table below).

| Test Concentration | 0mM | 100mM | 200mM | 300mM | 400mM |
|---|---|---|---|---|---|
| Ethanol Stock (500mM, or 3%) | None (add 25µL M9) | 10µL of 500mM | 20µL of 500mM | 30µL of 500mM | 40µL of 500mM |
| Worms (M9) | 25µL | 40µL | 30µL | 20µL | 10µL |
| Final volume | 50µL | 50µL | 50µL | 50µL | 50µL |

4. Carefully put a coverslip over the depression. Coverslip should seal, roughly, by suction. **DO NOT nail polish mount these.** Worms should be in solution for 1 minute before starting your video recordings.
5. Using the **compound microscope**, find your worms and focus in on your worms.
6. To count thrashes, refer to Figure 11. One thrash is when the worm moves from one side, crossing a midline to the other side, and back. **Record the number of thrashes for 15 seconds, for 5 worms.** Then, wait 10 minutes and do the same for the same slide. Use the table below for video recording times.
4. To count, you can do this manually or use a video. Use the cell phone camera and mount to acquire videos of your samples. Be sure to note the magnification you used, and get scale bars (4x or 10x might work best). For your notebook, you should have pictures with scale bars of each timepoint and condition. If you did manual counting, you still need pictures.
5. After completing the control slide, wash and dry your depression slide for reuse.
6. Make a new slide by pipetting a combination of M9 with worms and ethanol onto a new depression microscope slide (see table above). **Each member of the group**

**should do one concentration. For example, if you are a group of four, you need to do the control and three other concentrations. Recommended concentrations include the 200mM and 400mM EtOH. Pick another one if needed.**

a. REMINDER* - prior to adding worms, give the bottom of your 15mL tube a flick/tap to mix up the worms, so you don't pipette too little or too many worms by mistake.

You group, collectively, should collect the following information for each concentration tested.

| Concentration _____mM EtOh | Worm 1 | Worm 2 | Worm 3 | Worm 4 | Worm 5 | Average |
|---|---|---|---|---|---|---|
| Manual counting or Video 1 (15 seconds) | | | | | | |
| Wait 10 minutes | | | | | | |
| Manual counting or Video 2 (15 seconds) | | | | | | |

**Notes**

* Each student should do one of the concentrations. For example, if you have 4 people in your group, you should have data for the control plus 3 test concentrations.

- In your LA notebook, there is an excel sheet for you to use to fill out the table below.

| Thrashing timepoint | 0mM | 200mM | 400mM | Other |
|---|---|---|---|---|
| 1 minute | 100.2 | 80.4 | 60.7 | 10.7 |
| 10 minutes | 90.3 | 60.9 | 10.1 | 10.3 |

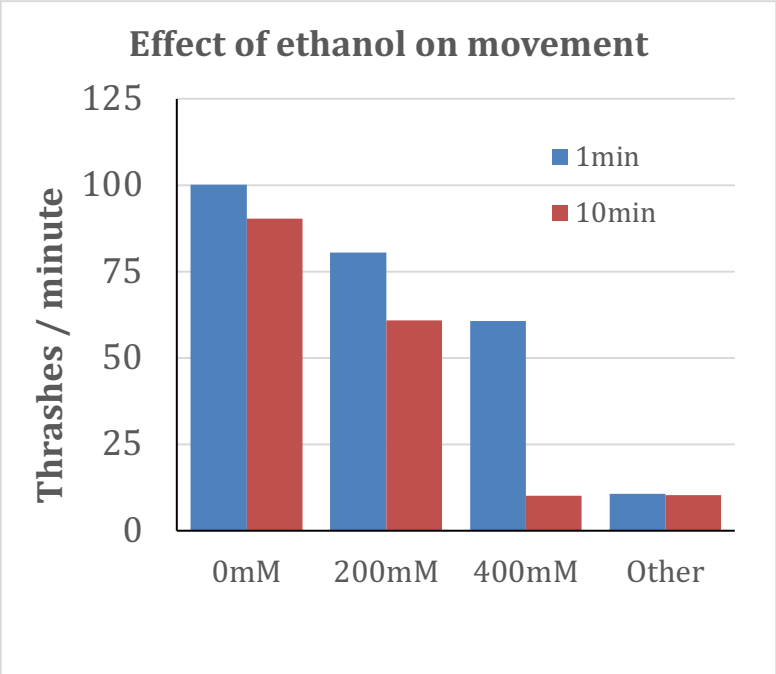
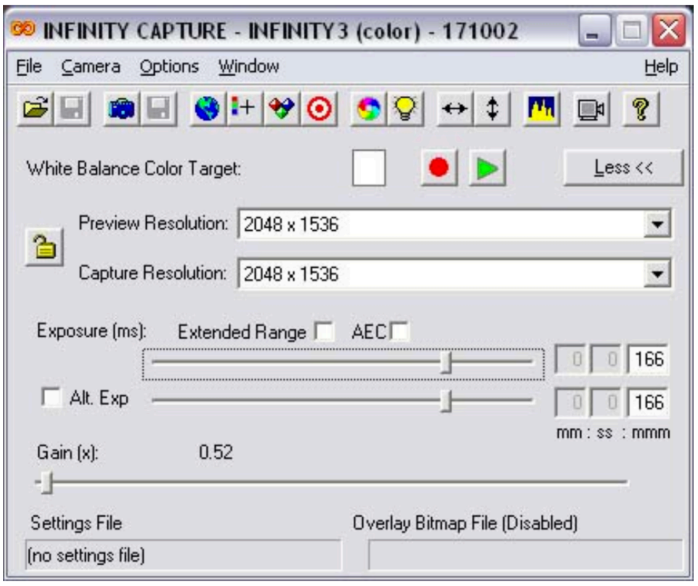



**Figure 15. How to graphically depict data showing the effects of ethanol on movement in *C. elegans*. A)** An image of how an excel entry might look like. **B)** A bar graph of example-*fake* data showing the number of thrashes per minute of worms in three different EtOH concentrations (other added if necessary).

**Infinity Capture (for taking pictures)**

1. Open Infinity Capture (located on your desktop). Once opened the following screen should appear:




2. DO NOT change any settings within the program itself, everything has been preset for you.
3. Once your image is focused (use your eyes, not the camera to get a rough focus of the worms), pull the shutter to switch the light to the camera (located on the upper right of the microscope). Re-focus as necessary.
4. First, you want to balance the light on your image. Do the white balance (globe button) to make the image more like what it looks like to your eyes. 
5. To take a picture, press the button that looks like a camera. 
6. Your image will appear. You must save this image. Go to **File > Save As > Save to the flash drive**
7. Remember to take a picture of the scale bar. You should elevate the scale bar to the same plane as the worms by placing the micrometer slide above a coverslip, before taking pictures.

## Day 4

### i. Day 4 BACKGROUND

There are many targets of ethanol, and this lab will explore one of the known cellular targets known as the BK potassium channel, called SLO-1 in *C. elegans*. SLO-1 is a $Ca^{++}$-activated K channel that limit excitatory neurotransmitter release in neurons of *C. elegans* (Figure 16). Normally, calcium enters through voltage dependent calcium channels (VDCC) and cause neurotransmitter release. Active SLO-1 channels lowers the activity to VDCC. Thus, active SLO-1 channels lower neuronal activity. Given that ethanol makes the SLO-1 channels more active, ethanol lowers neuronal activity as well.

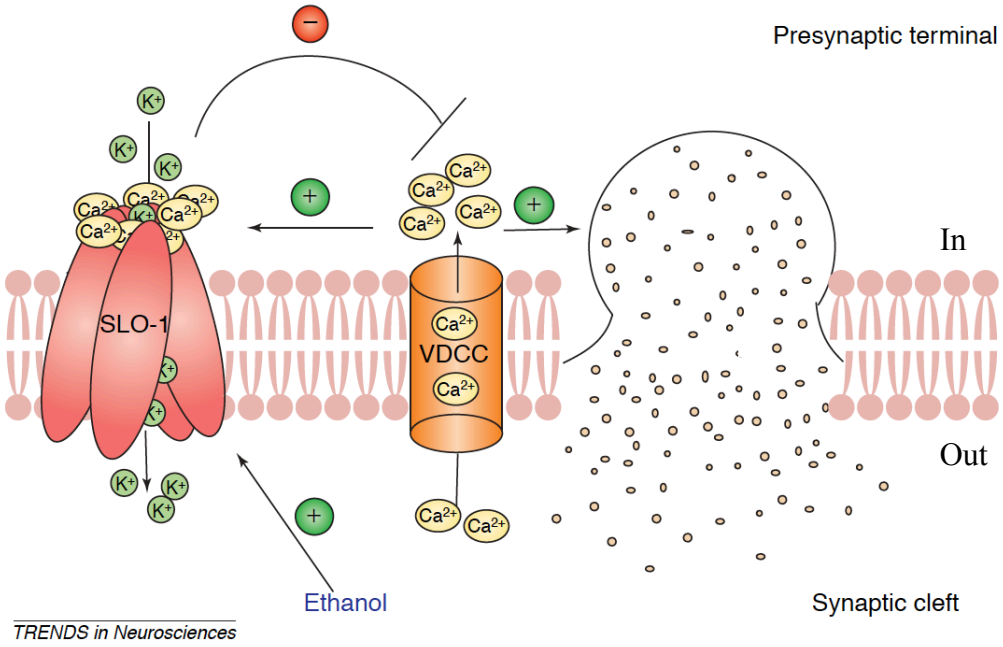



**Figure 16.** The effect of SLO-1 channel on neurotransmitter release. Figure from reference [6].

If SLO-1 channels are important for ethanol's effect on movement, then we would predict that animals having a mutation in the slo-1 gene would be less sensitive to the effects of ethanol. In this lab, you will use the procedures you learned previously to test this prediction.

## ii. Day 4 PROCEDURES

Exercise 1: Mutant learning movements on ethanol—(same sample size rules apply).

1. Now that you have had practice with some microscopy techniques, you will repeat your Day 3 experiments using mutant worms. These worms contain a mutation in a receptor that is thought to be a target of ethyl alcohol. It is called SLO-1, which is a potassium channel.
2. Repeat exercises 2 & 3 from Day 3 to see how this mutant compares to wild type in your experiments. **(your lab entry must have the combined data)**

Exercise 2. Data analysis

1. Associative learning
    a. See Day 3 for tutorial.
    b. Make graphs appropriate for the data.
2. Thrashing
    a. See Day 3 for tutorial.
    b. Make graphs appropriate for the data.

Exercise 3: Discuss your experiment and complete LA worksheet (**due at the end of class**).

1. As a group, develop a hypothesis around a question of interest. Begin outlining up your experiment by completing the science inquiry worksheet.
2. Things to remember…
    a. Carefully plan out your experiment
    b. Have a clearly defined variable(s)
    c. Use controls
    d. Make sure there is sufficient sample size
3. Come up with a list of materials you need to accomplish your experiment (which instructors will use to make sure you have everything you need).

Exercise 4: Discuss posters

1. Start preparing posters (organize, outline, plan)

## Day 5

### i. Day 5 BACKGROUND

Scientific communication is important for any aspect of biology, including research, medicine, drug discovery, and others. If your audience does not understand your data and your points, then your experiments, no matter how great, will lose impact. In particular, progress in science and medicine relies on people sharing ideas and disseminating information. Thus, the last two days of your module will aim to strengthen the accuracy and dissemination of your data to an audience.

To communicate our data for this module, you will make a poster and present it to the class. The poster will not be printed, but rather projected, and your group will talk through the poster. The parts of the poster are (see below for more information):

- Introduction
- Research Design
- Results (body size and brood size)
- Conclusions
- Future Directions
- Literature Cited

We expect you to be able to complete experiments in 2-3 hours. Afterwards and during downtime, we expect that you will work on your poster, and for us (Instructors and TAs) to work with you to make a great poster.

### ii. Day 5 PROCEDURES

Exercise 1: Perform your experiments

1. Collect data
2. Finish analyzing data
3. Prepare poster

**POSTER**

As a group, you will make a poster to show your data. See poster infographic and guidelines for more instructions.

**Poster guidelines**

I. Parts

Title and Authors

- Come up with clear, succinct and informative title (not BioLab Poster).

Abstract

- The is a brief summary of the whole poster

Introduction

- This section includes the background information on the research area and why your research is important
- Topic sentence followed by supporting sentences (can be 3-4 bullet points, but points should still be full sentences)

- Concise summary of your findings (same as abstract points), including your **hypothesis**

Methods/Research Design

- This section should describe how you set up the experiment?
- You can use a pictorial flow chart, or a bullet list of the *essential* points
- Microscope, camera, software you used
- Statistical/data analysis methods you employed, if appropriate

Results

- **Must have:** Representative images of all experiments, with scale bars.
- **Must have:** 1 graphs and table, with clear, units, legible
- **Must have:** Figure legends (the averages and sample size should be in the legend, along with a brief description of the data)
- Consider the quality of your images here (this is a microscopy class, after all!). If you have an image that has 10 animals, of all ages and sizes, which one are you actually trying to emphasize? Can you make the images meaningful to the audience?

Conclusions & Future Directions

- What conclusions can you make from your findings? (bullet point sentences are ok)
- What experiments can you propose to follow up?
- What is the relevance of this work?

References and acknowledgements

- Cite any references you used
- Acknowledge anything or anyone you want

II. Overall aesthetics

- Is there organization in the poster (topically)?
- Is there good formatting to the poster?
- Does the poster show any creativity?
- Is the content understandable?

III. Poster general advice

- Keep text to a minimal, and eliminate extraneous material
- Make contents visible from a distance
- Minimize distracting information (random colors or clipart)

## Day 6

### iii. Day 6 BACKGROUND

Oral scientific communication is an important part of being a biologist. You must not only know the material, but be able to explain it to your audience. Furthermore, you should understand your entire project, not just parts of it. Thus, each member of the group is expected to know all the parts of the poster, and NOT work solely on one or two parts. Work as a team to fill in all these sections. We have allowed most of Day 5 for you to do this, and for us (Instructors and TAs) to work with you to make a great poster.

### iv. Day 6 PROCEDURES

1. Load ppts onto Lab Archives by the start of presentations (10am or 3pm)

**(2)** Presentations will begin, and presentation grades will be peer reviewed.

## Appendix

### i. Lab notebook "rubric"

All lab notebook entries will use the following rubric. Each day will be given 6pts, or 6% of your module grade.

**Lab Notebook Expectations**
(note, this is the same or similar to what you learned in other biology and chemistry labs)

An important aspect of scientific work is the recording of all methods, observations, and data in a lab notebook. The biology and chemistry instructors at Juniata have come together to develop these notebook guidelines that you will use for a variety of introductory courses. Some of these guidelines may be geared towards chemistry labs while other aspects may be more suited for biology labs. Use your judgement as to what type of information is pertinent to your course, but when in doubt ask your instructor. ***Always bring your notebook to each lab and make entries as experiments are carried out.*** This document provides expectations for the information that should be included in every lab notebook entry (for coursework or research). Your instructor may make modifications to the below expectations for your particular lab (such as make certain sections optional). Some labs may also require that you include additional assignments as part of your notebook (such as pre- or post-lab assignments).

**Notebook grading policies:** Your instructor will review the grading policies specific to your course. (see rubric below)

**Importance of keeping a lab notebook:** A lab notebook may play several important roles. There are specific guidelines about what to include and how to format a notebook so that it can be used later. A lab notebook may:

- help you interpret your results (e.g., observations you thought were irrelevant at the time may be critical to later analysis),
- help you remember the experiment details, which is useful for repeating the experiment, and
- act as a legal document (e.g., patents, publications, enforcement of environmental regulations, etc.).

**What type of information should appear in my lab notebook?** Your notebook should contain the information necessary for someone to go into the lab and do exactly what you did. Critical data to include are *amounts*, *times*, *temperatures*, *results*, etc. But, a lab notebook that *only* records this information is useless. The lab notebook should be a narrative description of what you did in the lab. It should be complete enough that you could write your final report by looking at your notebook without looking at the lab instructions. Even better, someone else should be able to perform the same work that you did just by reading your notebook.

**What type of notebook should I use?** Your instructor will review the type of lab notebook that is required for your specific course. In general, hand-written notebooks should use a hard covered or spiral notebook with bounded pages (no loose-leaf notebooks). **This lab will use electronic lab notebooks (ELNs) system called LabArchives.** When using ELNs, be sure to **SAVE OFTEN**.

**Notebook safety:** Be very mindful about what you have on your hands when you touch your pen/notebook or your computer. It is not a good idea to write/type with gloves on, as this could spread microbes and chemicals.

**Getting started:** Use the first few pages of your notebook for the Table of Contents. Update the Table of Contents as you add information to your notebook, and be sure to reference the page numbers of your entries.

**Things to include in each entry (start each lab session on a new page):**

Lab notebook entries should have the following parts and answer the following prompts.

1. **Name (and collaborators if appropriate)**:
2. **Date**:

Today's data, or possible a range of dates if your entry spans multiple days.

3. **Purpose**:
    a. This is a brief statement about your day's goals, including biological and technical goals. It may be in the form of a hypothesis, or it may simply be a goals statement.
4. **Methods**:
    a. This section needs to provide enough information to repeat the experiment, but be succinct (bullet points are ok). It should be a past tense description of what you did, and SHOULD NOT be a complete copy and paste of the protocol.
    b. You should include important details, like: what was the Name of the microscope you used (Nikon Alphaphot-2), what objective did you used, how long you washed your mouth, what was the imaging software called, etc.?
    c. Examples of things we do not need are: I drew a line on my tube, the TAs gave us each a 15mL tube for washing, I named my jpeg file name_cheek cell, etc.
    d. If you are unsure, ask.
5. **Results**:
    a. You need to include a short paragraph summarizing your experimental purpose, findings, and overall results.
    b. You should include a figure (jpeg*) of your images (for example, cheek cells before and after the ethanol wash). Images should be **clearly labeled by treatment**. Be sure to include scale bars in your images. Consider the **quality** of the images you upload. Remember, this is a class on learning to use a microscope and communicating your findings. Select the best images you have. Consider adding an arrow(s) indicating which cell(s) you quantified from that image. *Note, imageJ can convert images from TIF to JPG. Go to your current image and File > Save As

c. You should also include a **labeled data table** that has all the raw data from your analysis, and include the mean cheek cell sizes.

6. **Discussion**
   a. Instructions are specific for individual labs. But you could address these common questions:

      How do your results support, or not support, your hypotheses?
      What do your results mean?
      How are your results similar to other published results, or other groups' results?
      What are some future experiments that can be done?
      What can you learn from microscopic analysis of your [the study]?
      Address reasons for abnormal results and technical difficulties.
      Address how your data is important to the general science topic.

**When you are finished in lab for the day:** Read through your entry and add to it as needed. If you made a mistake, strike through the error and put the correct information in [brackets] with your initials.

**Other standard notebook practices:**

- Write in blue or black ink (no pencil!).
- Put one line through mistakes (do not erase mistakes), and then put the correct information in [brackets] and initial the changes.
- Do not remove any pages from your notebook.
- If you copy information from someone else's notebook (e.g., your lab partner), be sure to indicate that the information was copied and from whom.
- Divide your lab notebook into left-hand and right-hand pages:
  - Use the left-hand pages for notes to yourself. This might include any special procedural things you want to remember, or information that you forgot to write earlier (draw an arrow to the place where it should have been entered). This is also a good place to do calculations and keep track of weights (e.g., weight of the container, weight of the container plus the chemical, weight difference). You can also use this space to remind yourself of any hazards that you may encounter (but note that you do not need to reproduce the entire MSDS or hazard analysis for every chemical or draw the hazard diagram for them).
  - The right-hand pages are for public consumption. This is where you write the information pertinent to the experiment you were doing. You should only write something down on the right-hand page after you DID it (use the left-hand page to write notes to yourself, such as how much of a reagent you plan to use, or your calculations for % yield) – after all, it may not happen the way you expect… Use the right-hand pages to record the amount of the chemical reagent that you actually used, and the actual % yield obtained.
- In some labs you may find it useful to make a table of the reagents you expect to use, their molecular weights, and a column for amount used, moles used, and equivalents used. You may also want to include some properties of your starting materials (such as melting points).

## Lab notebook rubric

| Item | Points | Full Credit | Half Credit | No Credit |
|---|---|---|---|---|
| **Names and Date** | 0.5 | There, easy to identify | | Not there |
| **Purpose** | 0.5 | Clear statement, including biological and technical goals | Makes some statement, but some parts unclear or not well stated | Mostly copy and paste from class tutorial, or states wrong information |
| **Methods** | (2 total) | | | |
| procedures in order | 1 | either written or bullet list of ALL specific steps (step dependent on lab) | Missed some steps, but got MOST of it | Steps wrong, or copy and pasted directly and didn't include lab specific details |
| language | 1 | past tense, clearly written | wrong tense, some unclear | Mostly copy and paste from class tutorial, or states wrong information |
| **Results** | (2 total) | | | |
| Figures (Days 2-4 only) | 1 | Great quality, shows use of Kohler technique, sizing, scale bar, etc. | OK quality, didn't use Kolhler technique or no/wrong scale bar | No figures, or poor figures (blurry, poor coloring) |
| Table (Day 2 and Day 4 only) | 0.5 | Table is easy to read, accurate, and includes sig. figs., and standard error | Most but not all of Full Credit info | Missing most of information |
| Talks about results (Figure Legend) | 0.5 | Provides description of figures and table, explaining the experiment well and REFERS the reader to look at particular parts of their results section. | Some description, but doesn't explain experiment or figure fully | No description |
| **Discussion** | (1 total) | | | |
| Comments on data (what result means) | 0.5 | Clear, well concieved discussion of the main experiment, and the results | Address main experiment, but doesn't explain well or is inaccurate | Misses the main points of the experiment |
| Explains abnormalities, unexpected results | 0.5 | Good explanation of any difficulties, and doesn't simply blame error on themselves, offers solutions | Some explanation, but doesn't offer fixes to the difficulties | Some explanation, but mostly blames "user" for problems |
| TOTAL POINTS | **6** | | | |

## ii. Poster guidelines

**Poster rubric**

| Item | Rubric (15) |
|---|---|
| **Title and Authors (1)** | 1 |
| | |
| **Introduction (3)** | |
| o This section includes the background information on the research area and why your research is important | 1 |
| o Topic sentence followed by 2-3 [bullet] points (if bullet points are used, they should still be full sentences) | 1 |
| o Concise summary of your findings (same as abstract points), including your hypothesis | 1 |
| | |
| **Research Design and Methods (3)** | |
| This section should describe how you set up the experiment? | 1 |
| You can use a pictorial flow chart, or a bullet list of the *essential* points | 1 |
| Statistical/data analysis methods you employed | 1 |
| | |
| **Results (body size and brood size) - (5)** | |
| o Representative images of all experiments, with scale bars. | 2 |
| o Graphs, clear, units, legible | 1 |
| o Figure legends (the averages and sample size should be in the legend, along with a brief description of the data) | 1 |
| o Consider the quality of your images here. If you have an image that has 10 animals, of all ages and sizes, which one are you actually trying to emphasize? Can you make the images meaningful to the audience? | 1 |
| | |
| **Conclusions and Future Directions (2)** | |
| o What conclusions can you make from your findings? (bullet point sentences are ok)<br>o What is the relevance of this work? | 1 |
| **Future Directions** | |
| o What experiments can you propose to follow up? | 1 |
| | |
| **Literature Cited (1)** | |
| o Cite any references you used | 1 |
| Total Score | 15 |
| | |

### iii. Poster presentation "rubric" for peer review

Here are possible questions the instructors and peer reviewers will use to determine your poster presentation (scale of 1-5). Your presentation grade will be determined by your review scores.

| **Poster presentation "rubric"** | | | | | | |
|---|---|---|---|---|---|---|
| **Poor:** Was not clear in explaining, didn't understand the images they used, didn't do any of the reading to understand material, didn't know answers to simple questions | | | | | | |
| **Average:** Did what was expected, explained just enough to understand the material and the data they presented | | | | | | |
| **Excellent:** Very clear, looks like they did beyond normal, including their own research and added to the interpretation of the data | | | | | | |
| Questions the instructors will use to determine your poster presentation | Poor | | Average | | **Excellent** | Your Group |
| Did the presenter(s) provide enough *background* information? | 1 | 2 | 3 | **4** | 5 | |
| Did the presenter(s) state the *purpose* and *rationale* for the experiments? | 1 | 2 | 3 | **4** | 5 | |
| Were the presenter(s) *clear* when they talked about their on results? | 1 | 2 | 3 | 4 | 5 | |
| Did the presenter(s) provide good *detail* in their results? | 1 | 2 | 3 | 4 | 5 | |
| Did the presenter(s) *relate their data* to topics well (diet/metabolism)? | 1 | 2 | 3 | 4 | 5 | |
| Were the presenter(s) able to *answer questions* when asked? | 1 | 2 | 3 | 4 | 5 | |
| Did the *group* work well together? | 1 | 2 | 3 | 4 | 5 | |
| Total Score: 35pts; scaled to 15 class points | | | | | | |

## iv. References

List of references

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3. http://www.microscopyu.com
4. http://wormclassroom.org
5. Mitchell et al. (2007). The concentration-dependent effects of ethanol on Caenorhabditis elegans behavior. *The Pharmacogenomics Journal* 7, 411–417.
6. Crowder, C.M. (2004). Ethanol targets: a BK channel cocktail in C. elegans. *Trends in Neurosciences,* 27(10): 579-582.
7. Mitchell, P. H., Bull, K., Glautier, S., Hopper, N. A., Holden-Dye, L., & O'Connor, V. (2007). The concentration-dependent effects of ethanol on Caenorhabditis elegans behaviour. *The Pharmacogenomics Journal*, *7*(6), 411–417. http://doi.org/10.1038/sj.tpj.6500440
8. Saeki, S., Yamamoto, M., & Iino, Y. (2001). Plasticity of chemotaxis revealed by paired presentation of a chemoattractant and starvation in the nematode Caenorhabditis elegans. *The Journal of Experimental Biology*, *204*(Pt 10), 1757–64. http://doi.org/10.1242/jeb.00433