Reference Modules

Reference

How Do I... ?

I. Equipment and materials at lab stations

This section provides a listing of all the equipment available—some will be available for every lab, some large items will be used for the entire class, others will be available at each station, some will be available for every lab upon request, and some will be available only for those labs in which it will be used. Note that this listing will change depending on availability of some seasonal items.

A. Items/materials available in every lab

1. Equipment:

Refrigerator
Fan
Timer (hour, minute, second)
SwiftCam
USB Camera
Swift Model 3200 microscope
Micropipettor (must request tips)
Ring stand with clamps
Scissors
Large Dishpan
Electronic balance (150 g capacity)
Microwave oven
Clip-on lamp (bulbs of various kinds available upon request BEFORE LAB)
Calipers
Spectrometer
Vernier Sensors (see Reference Module 4 part IV for a complete listing)
Ruler
Small misting bottle
Mortar and pestle
Graduated cylinders

2. Computer programs

Microsoft® *Word*
Logger Pro
Microsoft® *Excel*
Internet Explorer
LOGAL Explorer

3. Miscellaneous

Deionized water

B. Items/materials available upon request

1. Equipment

Digital Thermometer
Beakers (various sizes)
Plastic wrap
Aluminum foil
Hot plate/stirrer
Flasks (various sizes)
Plastic resealable bags
Safety goggles
Pipette pump with pipette
Eyedroppers
Heating pads
Latex-free gloves

2. Miscellaneous

Ice

II. Frequently asked questions about how to do stuff in lab

You will be collecting many different kinds of data in your laboratory investigations. The prelab assignments and reference materials should be especially helpful to you in designing your lab investigations week by week. However, you may have a question as you develop your plan of investigation that is difficult to track down using the standard table of contents. We have developed the cross-referenced list below to help you answer questions about measurement, data collection, and data interpretation. If you think of any other questions that should be added to this list, tell your lab instructor.

How do I...

Reference

Computer Basics

I. Using *Windows*

This section provides a very brief overview of how to use Windows so that you can access the software you will need for lab. If you want to know more, you might want to invest in a primer.

A. Keypresses

When you read the instruction "Click on the…", this refers to pointing to the object with the mouse pointer and clicking the LEFT mouse button. When you read the instruction "Right-click on the…," this refers to pointing to the object with the mouse and clicking the RIGHT mouse button. When you read the instruction "Press CTRL-A" this refers to pressing and holding down the CTRL key (lower-left or lower-right corner of the keyboard) and pressing the A (or other designated character) key. Similarly, when you read the instruction "Press Alt-A" this refers to pressing and holding down the ALT key (on either side of the space bar) and pressing the A (or other designated character) key. The Esc key is in the upper-left corner of the keyboard.

B. Main screen

Once the computer is started, and if no programs are running, you will see the "main window" for Windows which is called the desktop. The icons (pictures) that you find on the desktop are connections that execute programs to perform various tasks or give you immediate access to information in files. Along the bottom of the screen is the taskbar and in the far left corner of it is the Start button.

1. Taskbar

The taskbar shows you what applications (programs) you have running. When you start a program (See Start button below), the program will open in a window and its name will appear on the taskbar as a button. Because Windows allows you to run several programs at a time, you will see several buttons depending on the number of programs you are running. The buttons will automatically resize so that they all will fit.

2. Running programs

a. **To start a program—**Click on the  button. You will see a menu appear. You now point to one of the choices on that menu and if it represents a sub-menu, that will appear. You then point to names on that one, and so on. If no sub-menu appears when you point to a choice, then that item is the link to a program or a data file (i.e. word processing document, spreadsheet, etc.). If you click on one of these links the program will start. This manual will indicate the path to a program as such:

Start  **> All Programs > Accessories > Calculator**

Which indicates that you should click  then point to or click on the entry **All Programs**, then point to or click on the entry **Accessories**, then click on **Calculator**. In this case, that will start the Windows Calculator. If you want, try it.

Reference

b. To switch between programs—When several programs are running simultaneously you can switch between them by either:

1) Clicking on the window of the program you wish to use

OR

2) Clicking on the button on the taskbar that corresponds to the program you wish to use.

You will find this handy during the lab. This will allow you to run the program collecting your data while using, perhaps, *Excel* to analyze other data, *Word* to write your report, and *Internet Explorer* to access the library. Be careful though, the computers may lock up if you try to do too many things at once. **SAVE OFTEN**.

c. To terminate a program—Most programs will have an exit button somewhere obvious, but the universal Windows exit button is an  in the upper right corner of the window. Click it and it will close the program.

d. Minimizing/Restoring—To close a window without exiting the program, you can click on the  in the upper right corner of the window. You can restore the program's window by clicking on the program's taskbar button.

3. Moving information around, within, and between programs

a. Select—In most programs you can position your cursor at some point (e.g., at the beginning of a sentence, on a picture, on a graph) and click on it to select it. If there is more than one item to select, either drag the cursor over them while holding down the left mouse button (usually for text) or left-click objects while holding down the shift key (graphs, images).

b. Cut—Once you have selected what you want, you can cut your selection out by either:

1) Selecting **Edit > Cut** from the menu bar at the top of the program

OR

2) Clicking on the 

This is different from deleting the material. In this case, Windows holds the material in memory until you cut something else.

c. Copy—This action does not delete the material from where it is, but it does place a copy in memory for later use. You perform it by either:

1) Selecting **Edit > Copy** from the menu bar at the top of the program

OR

2) Clicking the 

d. Paste—Once you have cut or copied, you can now place the material somewhere else. You can paste it somewhere else in the same file or you can move material between files. This will be very valuable for placing illustrations or graphs in your lab reports or copying data between data collection, data analysis, and word processing software.

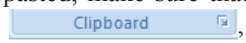
To paste material:

1) Select a spot by clicking at the point where you would like to insert the material. Then either:

2) Select **Edit > Paste**

OR

3) Click the 

e. Clearing the Office Clipboard—Once you have pasted, make sure that it is the correct data. If not, go to **Home Tab > Office Clipboard** 

, and select **Clear All**. Then, return to the previous program and try to copy and paste again.

f. Using Print Screen to capture a picture from the screen.

1) Press **Print Screen** on the keyboard. This will capture a picture of the entire screen and place it on the "clipboard".

2) Select **Start**  **> All Programs > Accessories > *Paint*** to open the ***MS Paint*** program.

3) Select **Edit > Paste** to paste the contents of the clipboard into *MS Paint*.

4) Select the portion of the image you want and **Copy** it.

5) Open *Word* (or another program) and **Paste** the picture into where you want it.

4. Working with files within programs

a. Creating a File—When you start many programs they will start a new file for you. When you need to create a new file from within your program, select **File** **> New**.

b. Opening a File—For some of what you will do in this course, we have created special files for you that will open automatically in the correct program. When you need to open another file or one you created, select **File**  **> Open** then choose from the menu.

c. Saving a File—You are allowed to save files temporarily so that your data will be safe while you are writing your report. **It is recommended that you save a newly opened document before typing anything. Stop and resave frequently.** To do this:

1) Select **File**  **> Save**.

2) If this is a new file, you will be prompted for a name—click in the box for the filename and replace the contents with the name you want for your file. Do not erase the extension, i.e. the "." and the three letters that follow.

3) Be sure you are saving in the default folder. Do not change where the file is saved.

4) If this is a file you have already saved, it will just be replaced.

II. Using *Excel 2007*

Excel is a program that creates a spreadsheet, which allows you to enter numbers (or text) into rows and columns and then manipulate them by entering simple formulas, analyze them by using a limited number of built in statistical tests, or graph them.

A. Starting the program

Click on **Start**  **> All Programs > Microsoft Office > *Microsoft Excel***

Reference

B. Organization

The screen in Figure R2.1 has a menu and tool bars [**A**] at the top and a series of tabs [**B**] at the bottom. These allow you to select actions and navigate around your spreadsheet. The bulk of the screen contains a lot of **cells** [**C**], i.e. places to put your data, which are organized into **rows** (1, 2, 3… down the screen), and **columns** (A, B, C… across the screen). Cells are identified by their column and row (A1 is the first cell in the upper left, A2 is the cell below it, B1 is the cell to the right of it, etc.). The tabs [**B**] on the bottom tell you which **worksheet** you are viewing. If you imagine that your file is a giant notebook, then the worksheets are pages in that book, each containing 256 columns and 16,384 rows. At the bottom of the screen is an optional toolbar of drawing tools [**G**].

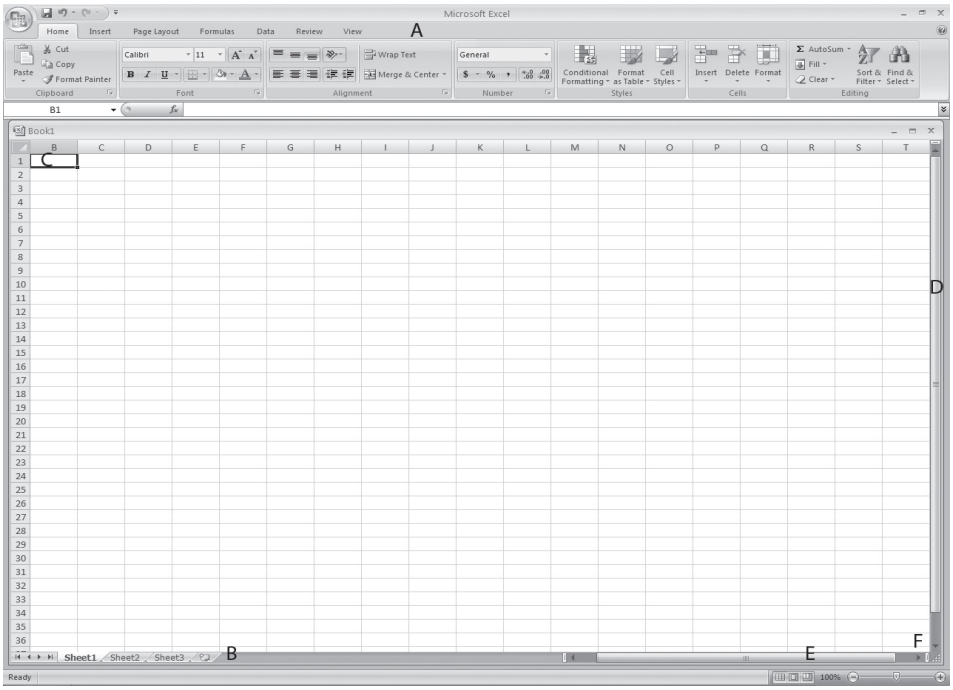


Figure R2.1 | Screen Features of a Typical *Excel Workbook*

C. Navigating

1. To see parts of the worksheet that are not visible, use the scroll bar [**D**] on the right of the screen to move up or down either by clicking on the arrows at top and bottom or moving the internal bar up or down; or use the scroll bar [**E**] on the bottom of the screen to move left or right either by clicking on the arrows at left and right **[F]** or moving the internal bar left or right.

2. To view a different worksheet, click on its tab [**B**].

3. If there are tabs for more worksheets than can be seen at once, you can scroll the tabs using the arrows in the lower left corner.

D. Making selections

1. Clicking on any cell selects that cell and makes it active.
2. Clicking any button on a tool bar performs that action.

3. Right-clicking on a cell or object brings up a menu of options or properties that allows you to change the characteristics of that item.
4. Pointing to anything on the toolbar for 2 seconds will cause an information box to appear with a 1-2 word description of the item.
5. To select a group (block) of cells:
   a. Click on a cell.
   b. Hold down the left mouse button.
   c. Drag the mouse in any direction—you will see the cells darken.
   d. Release the left mouse button.
   e. To add more cells to that selection:
      1) Hold down the Shift key and repeat 1-4 to extend the block continuously.
      2) Hold down the Ctrl key and repeat 1-4 to select an additional, non-contiguous block of cells.

E. Entering data

1. Click on any cell to select it and make it active.
2. Type to replace the content of that cell.
3. You can see what you are entering in the cell and also in the formula bar **[H]**.
4. When you are done, press the Enter key, or click outside the cell.
5. Use the Backspace key to erase errors before you finish.

F. Editing data

1. Double click on a cell or select a cell (as above) and click in the formula bar.
2. Enter text where you wish. Use the Backspace key or Delete key to erase.
3. To erase all the cell contents, click on the cell and press Delete. Do not erase content by using the Space Bar—*Excel* interprets any text as a zero value not a blank.
4. You can also **Cut**  , **Paste**  , or **Copy**  text.

G. Moving cells

1. To move the contents of (a) cell(s)

   a. Select the cell or cells.
   b. Select **Edit > Cut** or click  on the tool bar. You will see a dashed line around your selection.
   c. Select a new position (cell).
   d. Select **Edit > Paste** or click  on the tool bar to place the contents in the new location.

2. To make a copy of the contents of (a) cell(s)

   a. Select the cell or cells.

b. Select **Edit > Copy** or click on the tool bar. You will see a dashed line around your selection.

c. Select a new position (cell).

d. Select **Edit > Paste** or click on the tool bar to place the contents in the new location.

H. Creating a Graph

1. Setting up the data

a. Place your data in adjacent columns with each column representing a variable (see Figure R2.2).

b. If you have an independent (manipulated) variable to graph, place its values in the left-most column.

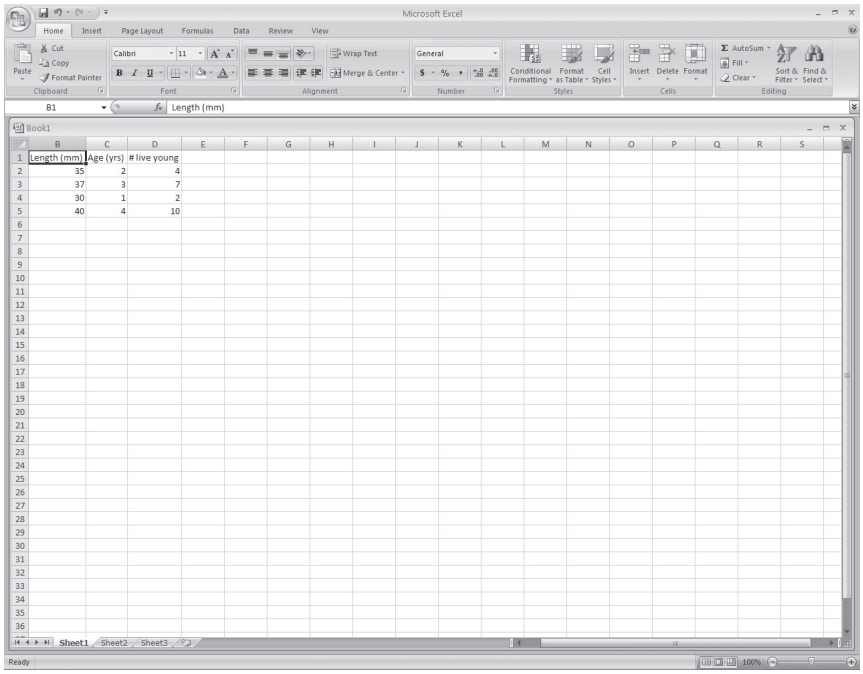


Figure R2.2 | Data Entered into Columns in a Spreadsheet

2. Selecting the Proper Graph

a. Select the block of cells that contain the data to be graphed.

b. Choose Insert from the menu at the top of the screen. When the menu opens up, choose the graph type that you want, then use the arrow on the icon of the graph to choose the graph sub-type.

There are many different ways to graph data. Each type is appropriate for different types of data and provides answers to different kinds of questions. In addition to the examples presented below, there are area, doughnut, radar, surface, bubble, stock, cylinder, cone, and pyramid graphs. Although you are free to choose whichever you think is best, it's a good idea to follow the KISS principle ("Keep It Simple, Student") here as elsewhere. This also true for embellishments like 3D effects-they are rarely necessary. The graph types in Table R2.1 are the most common and follow the naming conventions used in *Excel*.

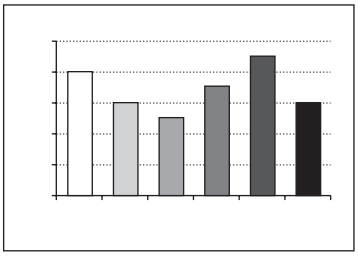
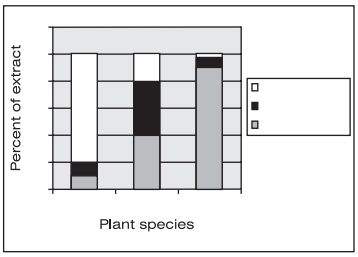
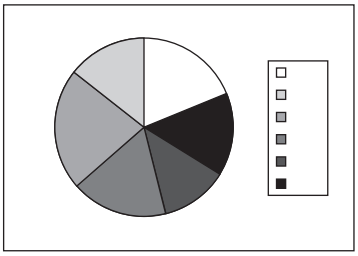
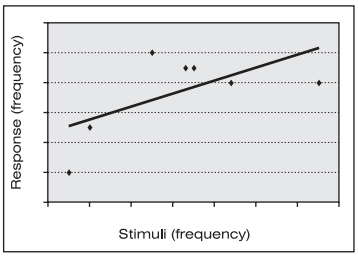
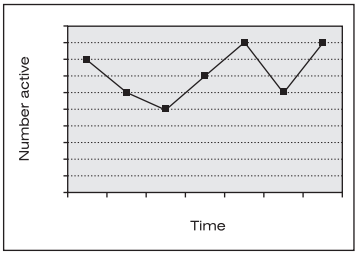
Type	Best Use
Column or Bar 	For discontinuous (e.g., <10, 10–20, >20), unordered or qualitative (male, female) data.
Column or Bar – Stacked 	For situations when you wish to express the part of the whole that data play (e.g., percent of the diet that four foods occupy for three species of fish).
Pie 	Contribution of each category to the total (e.g., proportion of a population of mice that are white, grey, or black).
X – Y Scatter Plot 	For continuous data. The data are pairs of X and Y values (although there can be additional Y's) representing an independent and one or more dependent variables. The data will appear as points that can be connected by lines. The data do not have to be sorted X-values (unless you choose to connect them by lines) and the X-values do not have to be evenly spaced. In this kind of graph, the points can be connected by lines, or a trend line can be added to indicate the best fit of a line to the data.
Line Graph 	Similar in use to X–Y for quantitative independent variable. However, X-axis is really categorical so the interval between must be uniform and data must be ordered.

Table R2.1 | Graph Types

3. Changing the data within the graph

a. Right-click to the side of the graph, but still within the border of the graph window. Choose **Select Data** from the menu options. –or- You can click the **Chart Tools** tab and then click the **Select Data** icon from the menu.

b. When the box opens the data that is displayed in the graph will be highlighted

c. To add more data to the graph (ie. Another Series), click on **Add** and specific which columns to include, which should be your X-axis data, Y-axis data and what the names of your data set (Series) should be in the legend

d. Click **OK** when you are finished

e. To edit data already displayed in the graph, click on **Edit**. This allows you to change data for both the X-axis, the Y-axis, and the Series name.

f. Click OK when you are finished

g. To remove a series of data, highlight the name of the series you wish to delete, then click **Remove**.

h. When you have finished manipulating your data click **OK**. If you do not click **OK** the changes you made will not be saved.

4. Adding Graph Features

a. Under **Chart Tools** on the top menu, choose **Layout**

b. This menu that opens up will allow you to add/change the graph title, the axis titles, the legend, the gridlines and other features of the graph.

I. Formatting a Graph

1. Changing Chart Characteristics

a. Click on the **Chart Tools** tabs at the top of the screen, and then choose **Layout**. You must have the graph selected in order for the **Chart Tools** option to show. This will allow you to change:

1) **Title of the Graph**
2) **Titles of the X and Y axis**
3) **Legend**
4) **Data Labels for each series**
5) **X and Y axis**
6) **Gridlines**
7) **Graph background**
8) **Trend line**

b. Click on the **Chart Tools** tabs at the top of the screen, and then choose **Design**. You must have the graph selected in order for the **Chart Tools** option to show. This will allow you to change:

1) **Chart type**
2) **Data in the graph**

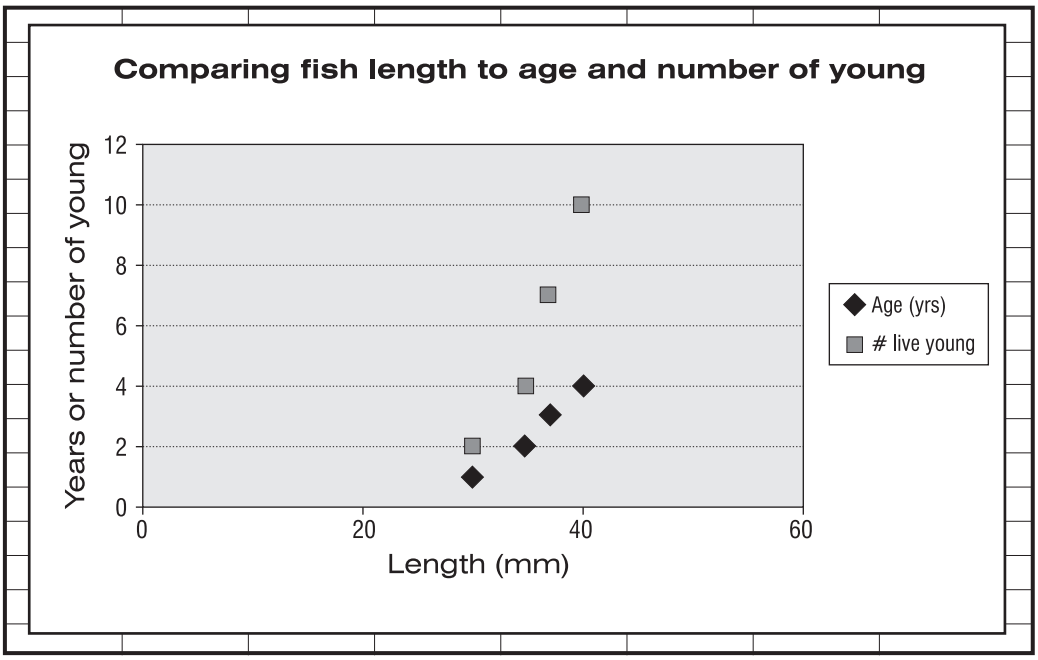


Figure R2.3 | Graph of Data From Figure R2.2

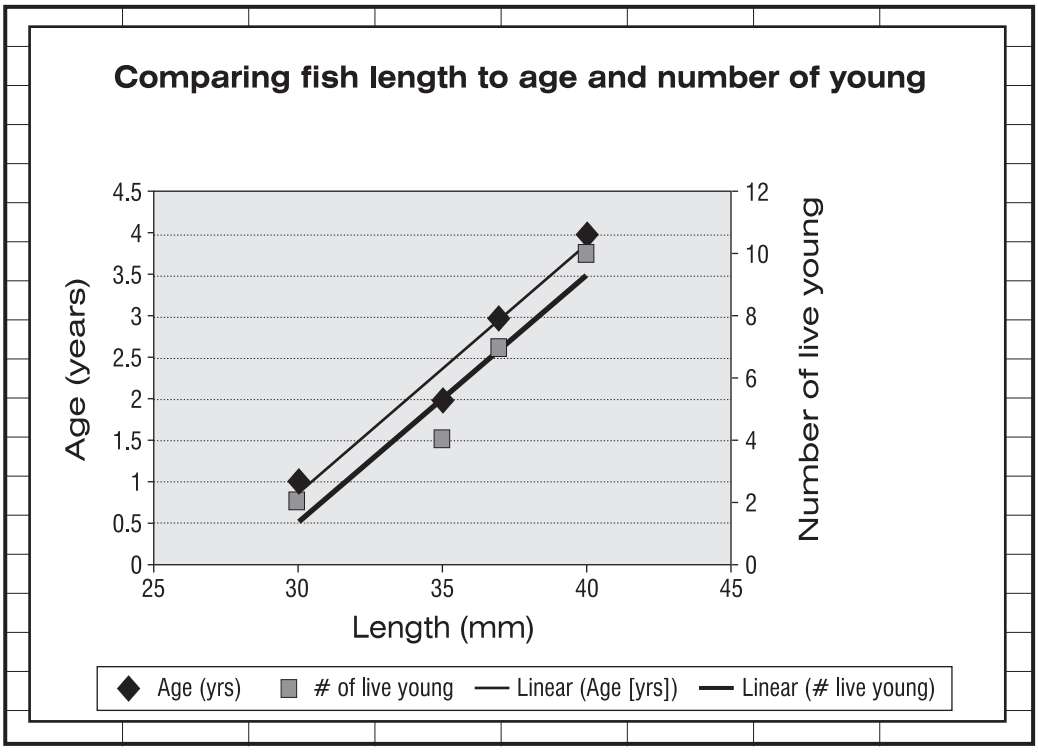


Figure R2.4 | Graph of data from Figure R2.3, showing optional trend lines and second Y-axis and a modified legend placed along the bottom.

3) **Style of the graph**

4) **Location of the graph**

c. Click on the **Chart Tools** tabs at the top of the screen, and then choose **Format**. You must have the graph selected in order for the **Chart Tools** option to show. This will allow you to change:

1) **The border of the graph**

2) **Word Art styles**

2. Adding a trend line to a scatter plot

a. Right click on a data point on the graph in the series you want to add the trend line to, and then click on **Add Trend line**. –or- Click the trend line icon located under the **Chart Tools** tab and then the **Layout** menu.

b. Choose the type of trend line-usually linear, otherwise you would have to explain why you thought another type of line made sense.

c. You can name your trend line or let Excel provide a default name which will be the same as the series name.

d. For the equation and R^2 value to be displayed on the graph, click on the box for both the equation and R^2 options.

Note: The R^2 value is an indicator of how well the line fits the data. A 0(zero) would indicate that the data points are randomly scattered around the trend line; a 1 indicates that all the points fall on the line.

3. Adding a Second Y Axis

a. First you must plot the data you want associated with a secondary axis on your graph as a new series.

b. Right click on a point from the data series that you want on a secondary Y axis.

c. Choose **Format Data Series**

d. Under **Series Options**, click on secondary axis

e. Click **Close**

4. Adding Data Labels

a. Right click on the series that you would like to associate with Data Labels

b. Select Data Labels from the menu.

J. Using formulas

If all you could do with spreadsheets was make tables and graphs, they would be of only limited use. The ability to create formulas in cells that use the data in other cells makes spreadsheets tremendously valuable. For example, a formula can take the content of one cell, multiply it by the content of another, and subtract a constant. By copying the formula to other cells, one can repeat the calculation many times. Using the many built in functions of *Excel*, you can perform many other manipulations, e.g., calculate the sum or average of a column of numbers. Some things you need to know:

1. References to cells are made by using their addresses.
2. Addresses can be absolute or relative:

a. When a relative address is used it signifies another cell based on its position relative to the one in which the formula resides.

b. Absolute addressing is used to refer to one specific location under any circumstances. This is signified by placing "$" before the row and/or column identifying e.g. "$A $1".

c. All formulas start with "=" so:

	A	B	C	D	E	F
1	Length (cm)	Width (cm)	Height (cm)	Volume (cc)	in^3	Conversion Constant
2	3.5	2	5	35	2.135	0.061
3	3	6	9	162	9.882	
4	4	1.5	3.2	19.2	1.171	

The formula entered into D2 is "=A2*B2*C2". When this is copied and pasted into D3, it automatically becomes "=A3*B3*C3". On the other hand, to convert to cubic inches in column E, each cell is multiplied by the contents of F2. The formula in E2 is "=D2*$F$2". When this cell is copied and pasted to E3 it automatically becomes "=D3*$F$2".

III. Using *Word 2007*

Your computer is equipped with Microsoft® *Word*. It is a writing tool and is capable of making tables and importing graphs or images from other programs (such as *Excel* or *Paint*). This software includes a spelling and grammar check.

A. Starting the program

Select **Start** > **All Programs** > **Microsoft Office** > **Microsoft® Office *Word***

B. To create a table in *Word*

Select **Insert** > **Table** > **Insert Table** as seen in Figure R2.5. In the **Insert Table** window, select the number of columns and rows you need. Then select **OK**. The table is then inserted into your document. To edit the table, click your curser in a cell and type data.

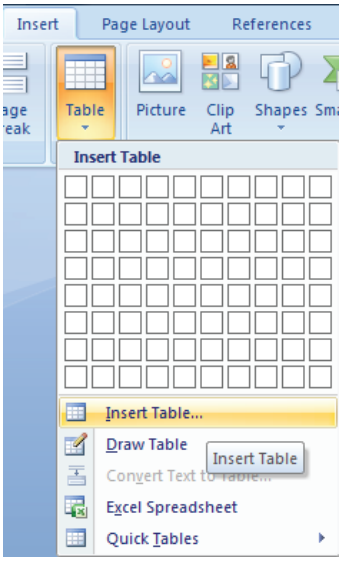


Figure R2.5 | Inserting a Table

C. To superscript or subscript

Using your mouse, highlight the character(s). Right click on the character(s) and select **Font**. Click in the box to select either superscript or subscript and select **OK**, or on the **Home** menu click the x_2 Subscript Icon or x^2 Superscript Icon. For example, CO2 becomes CO_2.

D. To insert a symbol

Click at the point in your document where you wish to insert a symbol. Under the **Insert** tab in the top menu, select the Symbol Icon Symbol and click the symbol you wish to add. For example, 37 degrees Celsius becomes 37° Celsius.

Reference

E. To learn more about using *Word*

Select **Microsoft Word Help** in the upper right hand corner. This opens the Word Help Window (Figure R2.6).

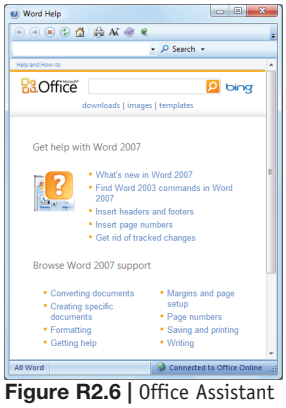


Figure R2.6 | Office Assistant

Using the Microscope

Written by Connie Russell

So, you think you know how to use a microscope. You might say to yourself, "Do I really need to read this section?" Before you decide that you can do without reading this, ask yourself the following questions: do I know what all the various parts of the microscope are used for? can I easily find the object(s) in focus? do I know how to change the amount of light focused on the viewing field? If you answered no to any of these questions then a quick review of this section may be useful (and time saving).

I. Overview

Microscopes (Figure R3.1) allow the user to view objects too small to be seen with the naked eye. Basically, microscopes provide magnification (make things appear bigger) and resolution (allow the viewer to see two objects that are very close together as two objects rather than as one). All the microscopes used in this lab are compound, light microscopes. Compound because they use a multiple lens system to magnify images, and light because images are viewed by the passage of light through the specimen.

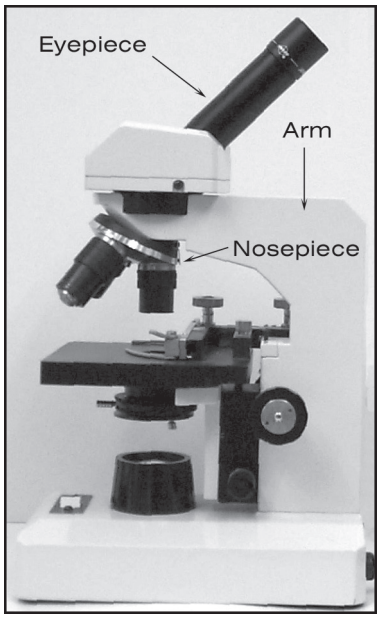


Figure R3.1 | Swift Model 3200 Microscope

II. Parts

A. Eyepiece (or ocular)

Contains a 10X lens. By itself, the ocular lens can magnify an object to appear ten times larger than its actual size. When looking into the microscope, the viewer should be able to see a graduated pointer. This pointer can be calibrated by placing a ruler on the stage and measuring the distance between the lines on the pointer at each magnification. The pointer can then be used to measure various objects in the field of view (see Reference *How to Measure*, II). The "head" that the eyepiece is connected to can be rotated 360° so that other viewers can observe the slide.

B. Arm (handle)

Contains the housing for the fine and coarse adjustments and connects the base of the microscope to the nosepiece and ocular.

C. Nosepiece

Rotating wheel that has the objectives attached to it (Figure R3.1). The objective to be used should "click" into position when the wheel is gently turned so that it is directly over the specimen slide.

D. Objective

Basically a housing for a lens (Figure R3.2). The microscopes in this lab have three objective lenses—4X, 10X and 40X (some have a 43X instead). Like the ocular lens, these lenses by themselves would magnify an object to 4, 10, or 40 (43) times its actual size.

E. Stage

(Figure R3.2) To be viewed, the specimen slide rests on this part of the microscope. The microscopes in lab have mechanical stages. Mechanical stages have knobs on the right side of the stage that allow the user to easily move the slide around on the stage. The slide sits securely between the L-shaped brace on the right and the movable sickle-shaped arm on the left. The knob closest to the slide moves the slide left to right, the other knob moves the slide front to back.

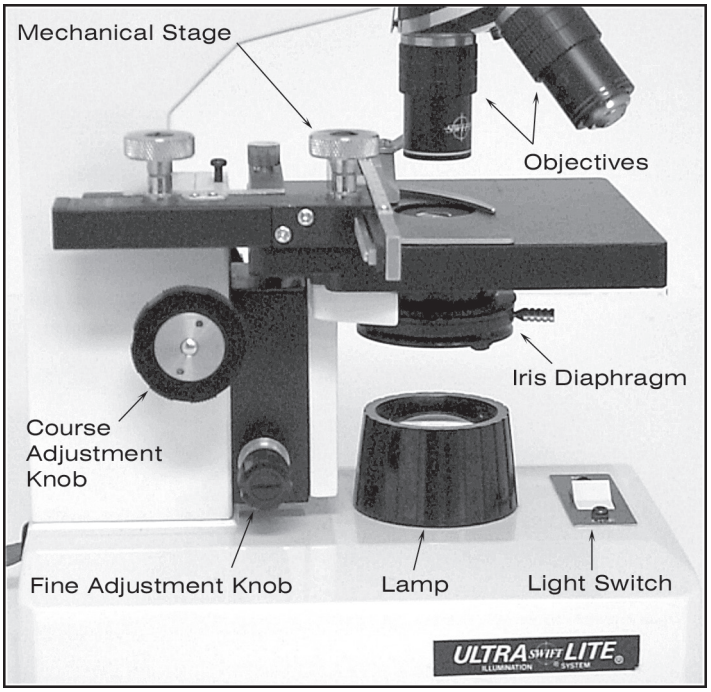


Figure R3.2 | Features D-J of the Swift Microscope

F. Coarse adjustment knobs

The larger of two sets of knobs located on either side of the arm just above the base (Figure R3.2). This adjustment is used to make large adjustments in focusing. It does this by moving the stage up and down. Because of this it is best to only use the coarse adjustment knob when the lowest power objective (4X) is in place.

G. Fine adjustment knobs

The smaller of two sets of knobs located on either side of the arm just above the base (Figure R3.2). This adjustment is used to make small (fine) adjustments in focusing. It can be used at any magnification, but is most efficiently used after focusing on low power with the coarse adjustment, then making small corrections with the fine adjustment before increasing the magnification. These microscopes are , which means that only small focusing adjustments are necessary when moving to a higher magnification if the object is in focus at a lower magnification.

H. Light source

Located directly under the stage (Figure R3.2). The on/off switch is located directly in front of it. If the light does not come on when the switch is in the on position, check to make sure that the microscope is plugged in. If so, ask the TA to replace the bulb.

I. Base

(Figure R3.2) The foundation of the microscope.

J. Adjustable diaphragm

This allows the user to adjust the amount of light that passes through the specimen. As a general rule, the lowest intensity of light that allows you to resolve the structure of the object you are viewing should be used. Too much light tends to "wash out" details, much like turning the contrast up on a computer monitor or TV screen "washes out" the images on them. There are two types of diaphragms on the microscopes in the lab—the iris and the disc. The iris dia-phragm (Figure R3.2) is controlled by turning a small lever located under the left side of the stage. To increase the amount of light, move the lever away from the arm; to decrease it, move the lever toward the arm. The iris diaphragm provides a finer degree of light intensity control. In general, you will need to increase the amount of light passing through the specimen as you increase the magnification. The disc diaphragm (Figure R3.3) has a wheel located under the left edge of the stage. There are five different settings—higher numbers correspond to more light. To change the setting rotate the wheel until it "clicks" into place.

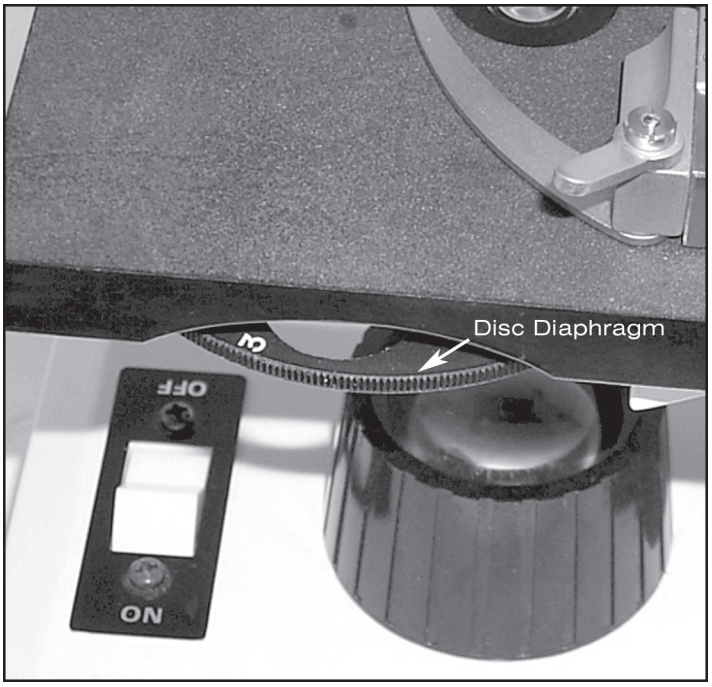


Figure R3.3 | Disc Diaphragm

III. Care and handling

A microscope is a delicate piece of equipment and should be treated gently. ALWAYS use two hands when moving the microscope from the cabinet to the benchtop. One hand should be placed around the arm of the microscope and the other should be supporting the base. It should always be carried upright and close to the body if it is to be moved any distance. The microscope should be placed on a flat surface, not too near the edge of the benchtop (where it might be knocked off). After you plug in the microscope, make sure that the cord is secured so that it will not be moved if the cord is accidentally pulled. If necessary, all the lenses on the microscope should be cleaned with a kim-wipe, cleaning with other materials (such as a paper towel) can scratch the surface of the lens.

When you have finished using the microscope, unplug the cord by pulling on the plug at the outlet—not by pulling on the cord. Wrap the cord loosely around the arm just above the base and secure the plug by tucking it inside the cord. Using both hands, replace the microscope in its designated space.

IV. Creating a slide

A. You will need a clean slide, a coverslip, and a sample source (pond water, a very thin slice of tissue, etc.).

B. Hold the slide as level as possible in your non-dominant hand or place it on the benchtop (Figure R3.4).

C. Place one or two drops of the sample in the center of the slide. For solid samples, place the sample in the center of the slide and add 1-2 drops of water or staining solution.

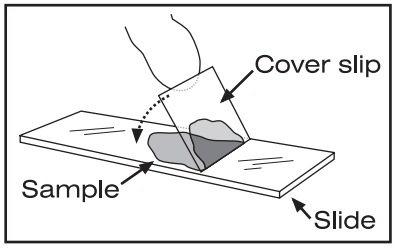


Figure R3.4 | Creating a Slide

D. Hold the coverslip in your dominant hand and place it close to the sample, without touching it, at a 45° angle. Slide the coverslip toward the sample until it contacts the liquid part of the sample. The liquid should spread along the entire edge of the coverslip. Slowly lower the coverslip over the sample. (Figure R3.4)

V. Viewing a slide

A. With the microscope setup in front of you ready for use, use the coarse adjustment knob to lower the stage as far as it will go. Rotate the nosepiece so that none of the objectives are in place. Secure your slide to the stage.

B. Rotate the nosepiece so that the 4X objective is in place. You should hear a slight "click" when the objective is seated properly.

C. Using the coarse adjustment knob, raise the stage as far as it will go.

D. Adjust the diaphragm to the lowest light setting.

E. While viewing the slide, lower the stage until you begin to focus on objects on the slide. When using the coarse adjustment no longer sharpens the focus, use the fine adjustment. Adjust the light as necessary.

F. Increase the magnification as necessary by rotating the nosepiece to place the next objective of higher magnification over the slide. Use the fine adjustment knob to sharpen the focus, making only small adjustments. If you lose the object you are trying to view or if you cannot get the object into focus, go back to the next lowest magnification, focus there, then attempt to increase the magnification again.

VI. Measuring objects

There are times when it is useful to know the actual size of the object you are viewing with the microscope. For example, some species of algae can be identified in part based on their size. The pointer on the microscope (Figure R3.5) has a scale embedded on it that can be used to measure objects under the microscope by carefully aligning the object with the tick marks on the scale and taking a linear measurement just like you would on any ruler. The actual size of the object is determined by dividing the scale reading by the total magnification.

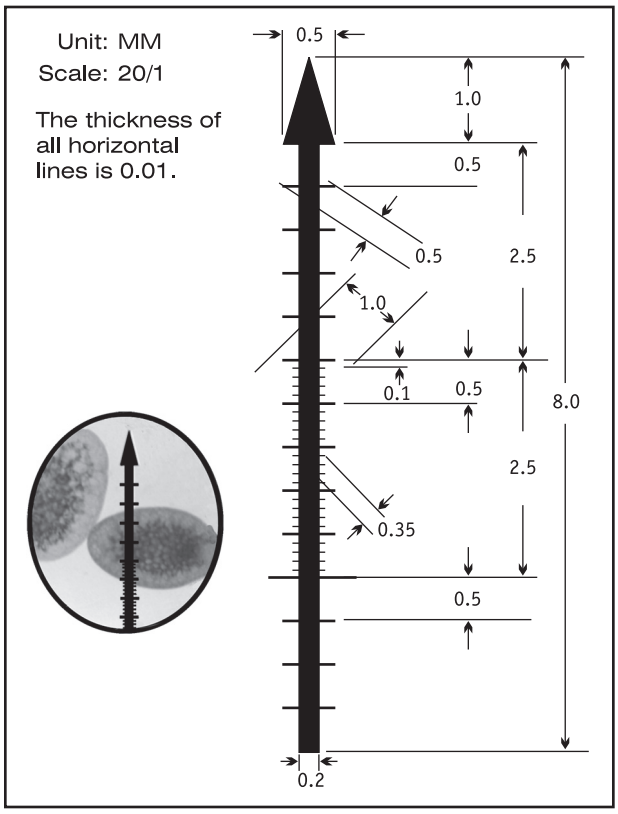


Figure R3.5 | Swift Microscope Pointer Scale

VII. General microscopy tips

A. You will strain your eyes less if you keep both eyes open while viewing a slide. This will seem awkward at first, but will get easier with practice.

B. As you increase the magnification, increase the amount of light on the specimen.

C. View objects on the lowest magnification that allows you to answer the question you are investigating—don't spend an inordinate amount of time trying to get a better look.

D. When making slides, less is more. Use the smallest amount of sample, stain, or tissue that is possible.

E. If you are using one of the SwiftCams to take a picture of your sample, focus the object of interest without the camera first—this will save you time in the long run.

Reference

Reference

Chemical and Physical Sensors

Written with help from Moria Harmon, Matthew Anderson,
Cassandra Walker, and Brian Decocq

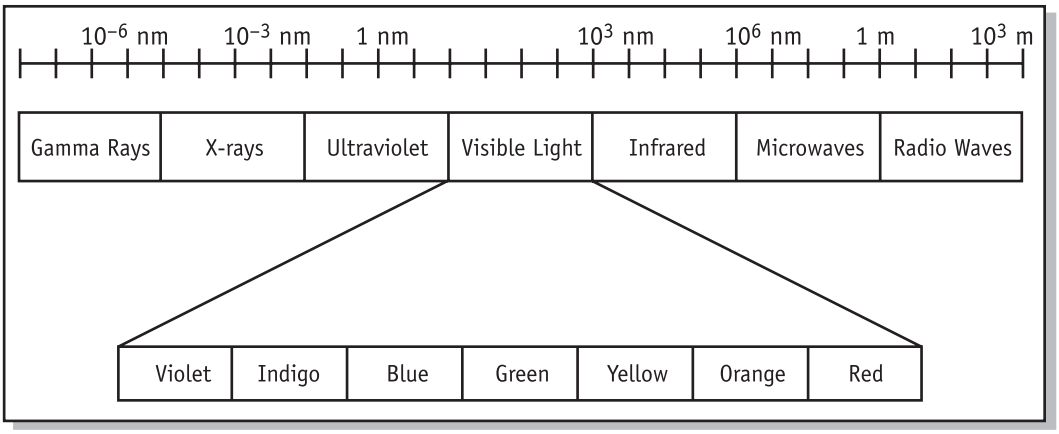
I. Spectrometer

A. Theory of operation

A spectrometer is a device that measures the amount of light passing through or absorbed by a solution. To do this it produces a tightly focused beam of light that passes through the chamber that holds the sample vial (cuvette) to a photocell. The particular spectrometer you will be using is capable of scanning a range of 380-900 nanometers (nm); this range includes the ultraviolet (<390 nm), visible (390-760 nm), and infrared (>760 nm). See diagram below.

The light that is absorbed by a particular chemical is precise and predictable. The amount that is absorbed will change with the concentration, but which wavelengths are absorbed will not. So if one graphs absorbance vs. wavelength for a particular chemical, the height of the "peaks" and "valleys" may change with concentration, but they will always be at the same wavelength. This means that if we desire to see what particular chemicals are in a solution we could scan the solution and compare that to known chemicals to determine the composition of the unknown solution.

When selecting your units to read, you will notice that one is percent transmittance and the other is absorbance. These units are similar to reflection versus absorption, in that transmittance measures the amount of light remaining or unused by the molecules in solution. Transmittance is a function of absorbance, that is, when the absorbance value is 0 then percent transmittance is 100%.



Reference

B. Controls

Although the Vernier Spectrometer (Figure R4.1) is small, it is a research grade instrument, capable of collecting measurements rapidly over a range of 380-950 nm. Using LoggerPro, it measures the absorbance spectrum of a solution and produces results that can be graphed and analyzed via LoggerPro or Excel. The Vernier spectrometer is connected to the computer directly via a USB cord. The spectrometer will display the Absorbance [abs] against one of three independent variable selections (wavelength [nm], concentration [mol/L] or time).

Figure R4.1 | Vernier Spectrometer with a cuvette.

C. General operation

1. The sample holder

The samples you measure need to be placed into cuvettes. The cuvettes are shaped to fit into the sprectrometer and should be oriented so the clear sides (sometimes indicated with an arrow) of the lower portion of the cuvette are inline with the light path. To ensure that you are measuring the true absorbance, the concentration of the sample must be low enough to allow light to reach the other side of the chamber where the detector is located (abs value of ≤ 2).

2. Startup procedure

a. Click on Start → Programs → Vernier Software → Logger *Pro* 3.4.5 to load the software.

b. From the Logger Pro main Menu Select Experiment → Connect Interface → Spectrometer → Scan for Spectrometers.

3. Zeroing procedure

All containers and solutions absorb light differently, so to use the spectrometer to measure the light transmitted through, for example, a purple dye that is dissolved in water, one must account for the water and the cuvette. This is done by letting the spectrometer measure the amount of light absorbed by the water and the cuvette without the dye and setting that value to zero. The clear liquid used to zero the spectrometer is referred to as the "blank". **For each new solution you must zero the spectrometer with a sample of the solvent used to suspend the pigment being tested.** If you close out the LoggerPro software, you must restart from the step titled Startup Procedure.

a. To zero the Spectrometer, from the LoggerPro main menu choose Experiment → Calibrate → Spectrometer.

NOTE: The calibration dialog box will display the message: "Waiting … seconds for lamp to warm up." The minimum warm up time is one minute.

b. Follow the instructions in the dialog box to complete the calibration.

Click *OK,* when completed.

4. Choosing a Unit of Measure

Prior to taking measurements, you must determine what your final output should be based on your experimental design. Is your independent variable time, concentration or the wavelengths that comprise the visual spectrum? Are you reporting your dependent variable as percent transmittance or absorbance?

a. Assigning correct dependent variable units

To select the unit of measure, from the Logger Pro menu choose Experiment → Change Units → Spectrometer. Click on the unit of choice from the list.

b. Assigning correct independent variable units

To select the unit of measure, from the Logger Pro menu click Configure Spectrometer Data Collection . Indicate your desired collection mode, i.e. your dependent variable and one of the three corresponding independent variables. Clicking OK.

5. Measuring a sample

To measure a sample for the experiment, remove the blank, and follow the procedures below. It is important to determine the range of wavelengths to be tested. Some hypotheses will address a very narrow spectrum, while others will examine the entire visible spectrum. To change the range of wavelengths tested, click Absorbance = 0.015. Select the range that you wish to test and click Close.

5. Measuring a sample

a. Fill the sample cuvette about ¾ full with the experimental sample.

b. Place the sample in the cuvette holder of the Spectrometer.

c. Click Collect

d. To stop collection, usually 5-10 seconds after starting, click Stop

e. Examine the Graph and note the region(s) of maximum absorbance. Peaks of ≥ 2 are errors generated by the spectrometer. If values ≥ 2 are reported you should reduce the concentration of the sample. Refer to Section on Serial Dilutions

f. To store the spectrum data, choose Store Latest Run from the Experiment menu.

g. To display a graph either select print from the file menu inside the Logger Pro menu or export the data collected to MS Excel and produce an appropriate graph for final presentation.

D. Alternative Experimental Designs

Some experimental designs will focus on a range of wavelengths that comprise a desired color of light, and others will address the entire visible spectrum. Using LoggerPro and the Spectrometer you may select as many wavelengths as you wish. There are three ways to select the wavelength(s).

1. Perform a Full Spectrum Analysis

This method is best when you wish to keep a copy of a full spectrum graph (Figure R4.1). Conduct the full spectrum of a sample of solution and examine the graph. Go to the Configure Spectrum Data Collection dialog box and select Abs vs. Time. The wavelength of maximum absorbance will be automatically selected.

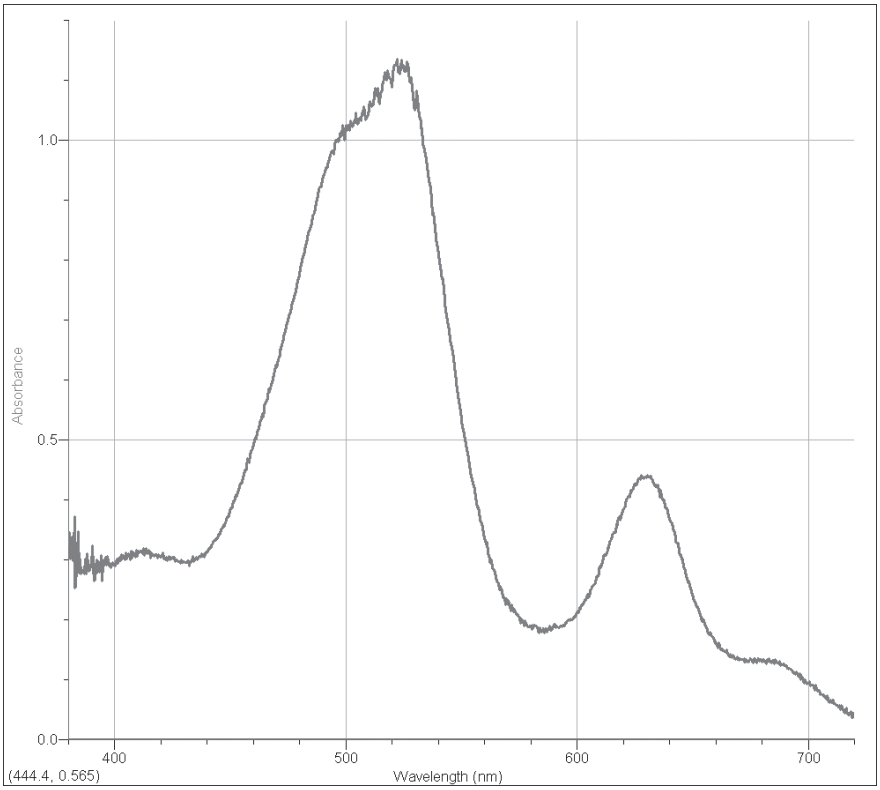


Figure R4.2 | Graph of absorbance across the visual spectrum of unknown solution

2. Determine Peak Absorbance

This is a test where the full spectrum analysis is not needed. After calibrating the Spectrometer, place a sample of solution in the Spectrometer and go to Configure Spectrum Data Collection dialog box. Select Abs. vs. Time for a Kinetics Experiment or select Abs vs. Concentration for a Beer's Law experiment. The wavelength of maximum absorbance will be automatically selected.

3. Select the Wavelength of Maximum Absorbance Manually

This method can be used when you already know the precise wavelength to be used in an experiment. After calibrating the Spectrometer, go to the Configure Spectrum Data Collection dialog box. Select a wavelength on the graph or in the list of wavelengths.

4. Selecting a Range of Wavelengths

In many experiments you may wish to measure the absorbance or %T of a sample over a group of wavelengths. From the Configure Spectrum Data Collection dialog box, you may select the wavelengths one at a time by checking the boxes in the Full Spectrum column or place the cursor on the graph in the dialog box by left clicking and dragging across the region of wavelengths that you wish to analyze. Make sure to check the "Treat Contiguous Wavelengths as a Single Range" box.

E. Things to watch out for

1. Always be sure to use the same solvent in the sample solution and the blank.

2. Be sure that the blank is filled to a point above where the beam of light will pass through it (~3/4 full) or you may get inconsistent results. This may seem obvious, but often students (or even researchers) forget or don't check.
3. Do not use a cuvette that is scratched or stained. Ask your TA for a new one.
4. Use a lint-free tissue to wipe off any finger prints from the cuvette. (Finger prints absorb or scatter light!) Hold the cuvette above the level of the solution.
5. CAUTION! Do not put solution directly into the spectrometer!

II. Using Vernier sensors

A. Overview of software

This software allows you to collect data from any of the probes available in this laboratory. These include Temperature, pH, Dissolved Oxygen, Light Intensity, Relative Humidity, Conductivity, Gas Pressure Sensor, O_2 Gas, and CO_2 Gas Sensor. You can control the display, type of data collected, duration of the experiment, number of data points collected per unit time (sampling rate), and the way you analyze the data. You are able to customize many different items and you may choose to do so to make the best of the experiments you have designed. However, as a starting point, the software has been set up for you in several configurations that match combinations of probes you might use.

B. Setting up the probes for use

1. Choose the sensor(s) you want to use. See section III for sensor descriptions.
2. If the probe(s) are not already inserted in your LabPro or LabQuest interface, insert them firmly (Figure R4.3). Make sure the sensor is connected to the input (channel) on the interface that corresponds with the sensor setup within the program. See Sensor Selection (II.C.13) for more details.

Figure R4.3 | Connecting a Probe to the Interface

C. *Logger Pro* software

1. Starting the software

Select **> All Programs > Vernier Software > {template}**

The {template} you would select depends on the experiment you are planning. Each template has been set up to record data from one or two probes and to display the information on the screen in an appropriate format.

2. Objects

When the *Logger Pro* software opens, you will view a screen similar to that in Figure R4.4. *Logger Pro* organizes your work in terms of "pages" (screens), on which are "objects" such as the ones described in this section. You can place whatever objects you want on each page, and then switch from page to page as described later. However, we recommend that you use the templates we have built and work with only one page.

a. Graph Object—This object (A in Figure R4.4) shows the data you are collecting in graphical form.

b. Table Object—This object (B in Figure R4.4) displays your data in a tabular form. Each row is a point in time and each column represents a variable. When you collect data, the values will appear here, row by row. You can scroll to view more data using the scroll bar at the bottom or right side of the object.

c. Meter Object—This object (C in Figure R4.4) is an enlarged display of the current values for the items being measured.

The program affords you much more control and many more options than will be discussed here. You are free to explore them, but remember that other people will be using this program, so do not make changes that will alter the program's future operation. If you are not sure if something is working properly, ask your instructor.

Options described here are of particular value to you.

3. Menu bar

The menu bar (Figure R4.5) at the top of the screen allows you to set up, run, display, and analyze the data from an experiment.

a. You are probably already familiar with the functions **New** document, **Open** existing document, **Save** current document, and **Print** screen.

b. To scroll between pages within your document, use Page 1. However, we recommend that you do not create new pages. See the **Page** menu below for more detail.

c. For quick access to the **Data Browser** window (details under the **Data** menu), you can use .

d. The x- and y-axis will scale automatically to fit your graph if you click .

e. You can zoom in at a selected region of your graph with . To zoom out click .

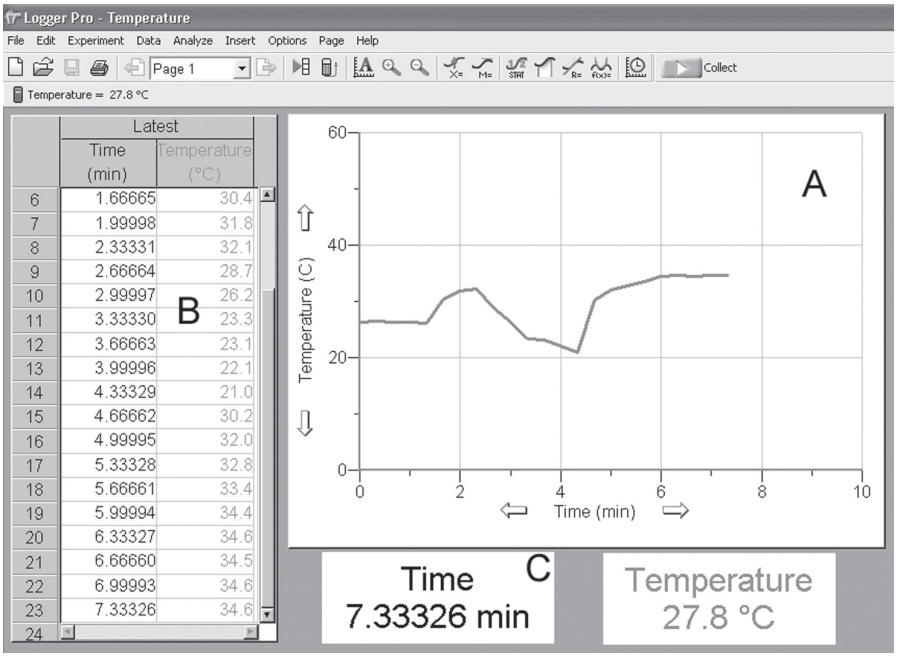


Figure R4.4 | Examples of *Logger Pro* Objects

Figure R4.5 | Menu Bar

f. Math functions appear in the toolbar as  . Those that you are most likely to be interested in are detailed in the **Analyze** menu.

g. To adjust data collection settings, use  . This function is described within the **Experiment** menu.

h. To begin data collection, use 

. To end data collection, use 

.

4. File

(Figure R4.6) Manipulates the entire document file.

a. **New—**This will open a new document. Unfortunately, it will open a new default file that will not be familiar to you. We recommend that if you need to restart collection or add new data, store your latest run (described in the Experiment section [II.C.6]) so that you may remain in the same file. Or, you can always open the file that you need with the correct probe setup from the **File** menu.

b. **Save—**Saves the current document using the existing file name.

c. **Save As—**Saves the current document to a specified file name.

d. **Printing Options—**Lets you add your name and other comments when you print your data. We recommend that you add your name on the page so you can quickly identify your graph.

e. **Print—**Lets you print your data table, your graph, or your entire document.

f. **Exit—**Allows you to quit the program.

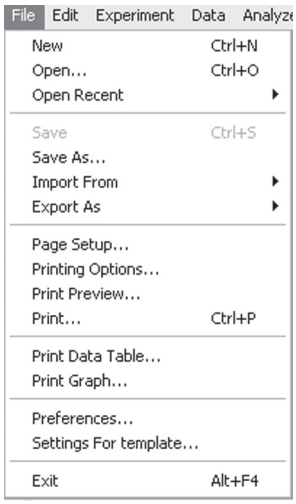


Figure R4.6 | File Menu

5. Edit

(Figure R4.7) Moves data within the document and between applications.

a. You may use the typical **Cut**, **Copy**, or **Paste** options to move data to other applications (including *Word* documents).

b. **Select All—**Lets you select all your data. Use this before a **Copy** or **Analysis** command.

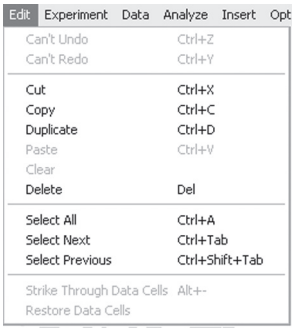


Figure R4.7 | Edit Menu

6. Experiment

(Figure R4.8) Set up for data collection.

a. Start Collection—Starts collecting data.
Same as 

.

b. Store Latest Run—Saves your most recent data allowing you to collect another data set without losing the first. The stored run will be moved to the far right of the data table but remain graphed. *This method is suggested for when you need to collect multiple sets of data rather than creating a new page.*

c. Data Collection—Modifies settings for your experiment. Same as  in the menu bar.

1. Collection tab
   a) Mode of collection – time or events based
   b) Length of data collection
   c) Rate of sampling
2. Triggering – Can be set up to collect data following a specific sensor value as a trigger.

7. Data

(Figure R4.9) Functions for clearing or storing data.

a. Data Browser—Shows all of the data sets and columns. Allows access to add a new data set, add a manually created column of data, and delete currently selected objects. This tool duplicates all of the data functions below.

b. New Data Set—Creates a new data set. This is the same as the **Store Latest Run** option.

c. Show Data Set—Show a hidden data set.

d. Hide Data Set—Hide the selected data set.

e. Delete Data Set—Select a data set to delete.

f. Delete Column—You may choose to delete a column(s).

g. Clear All Data—Clears all the data in the current page.

8. Analyze

(Figure R4.10) Manipulates and summarizes data that have been selected.

a. Statistics—Calculates the minimum, maximum, mean (average) and standard deviation of the selected data (e.g. what is the mean temperature during minute 2?). Can also use

.

b. Linear Fit—Draws a straight line in the graph that most closely approximates the trend in the selected data (i.e. rate of increase or decrease). Provides the slope and y-intercept of the line. Can also use 
.

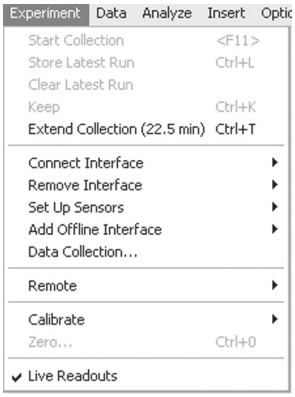


Figure R4.8 | Experiment Menu

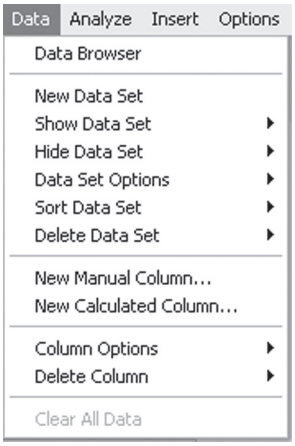


Figure R4.9 | Data Menu

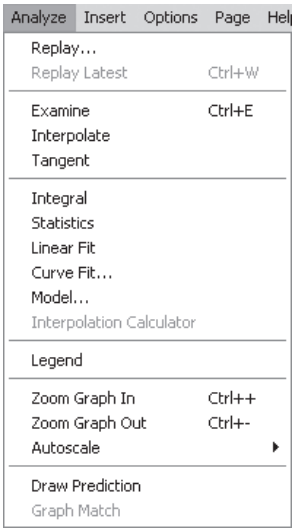


Figure R4.10 | Analyze Menu

c. **Curve Fit—**Draws a curve through the data based on the function you select. Can also use . For more information, see IV.G within this section.

9. Insert

(Figure R4.11) You can insert new graphs, tables, and other additional objects into the current data.

a. **Meter**

1. **Digital Meter—**Adds a new digital meter to display the current data reading. You can click and drag to move the meter window. To change which data the meter displays, right click on the meter, select **Digital Meter Options**, go to **Column**, and select the variable from the drop down menu.

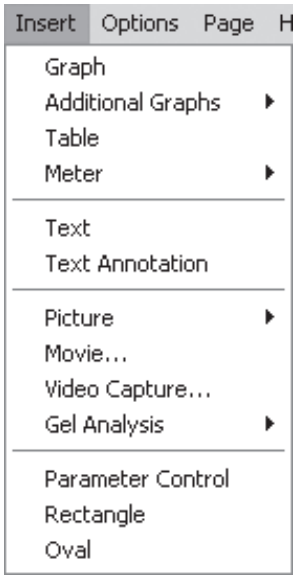


Figure R4.11 | Insert Menu

10. Options

(Figure R4.12) Objects (graphs, tables, meters etc.) can be edited.

a. **Graph Options—**Allows you to change the appearance of the graph (such as the scale of the x- or y-axis).

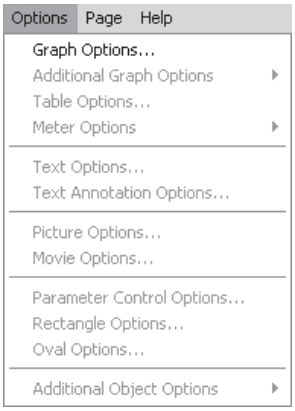


Figure R4.12 | Options Menu

11. Page

(Figure R4.13) Allows you to create and modify new pages within a document and move from page to page. A page allows new data collection within the same document. However, if you select to add a new page the design will be a default setting that will be unfamiliar to you. Again, we suggest that you store your latest run or reopen the program.

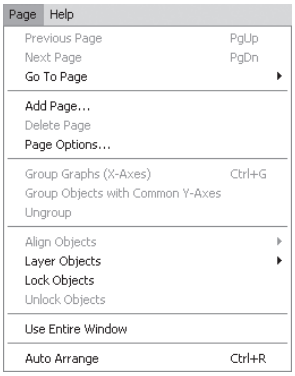


Figure R4.13 | Page Menu

12. Help

(Figure R4.14) Provides extensive information about the functions of the program. Go to ***Logger Pro* Help...** for quick access to menu descriptions, how to, troubleshooting, etc.

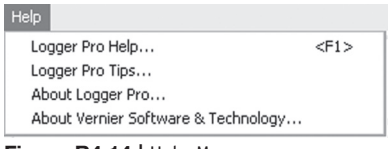


Figure R4.14 | Help Menu

13. Sensor selection

Allows you to select a sensor or combination of sensors from a menu. To access the Sensor Selection Window (Figure R4.15), select **Experiment > Setup Sensors > Show All Interfaces.**

To select a sensor, scroll through the menu on the left then click and drag the icon of the desired sensor to the box that corresponds to one of the inputs (channels) on the LabPro interface. For example, in Figure R4.15 you can see that the temperature probe has been selected for CH1 (channel one). For the probe to work properly, it must be connected to CH1 of the LabPro interface.

To remove a sensor from a channel, right click on the icon of that sensor and select **Remove Sensor**.

You will find it easier to use the probe combinations that are already provided for you. However, selecting your own combinations may be useful if you have a particularly creative experimental design.

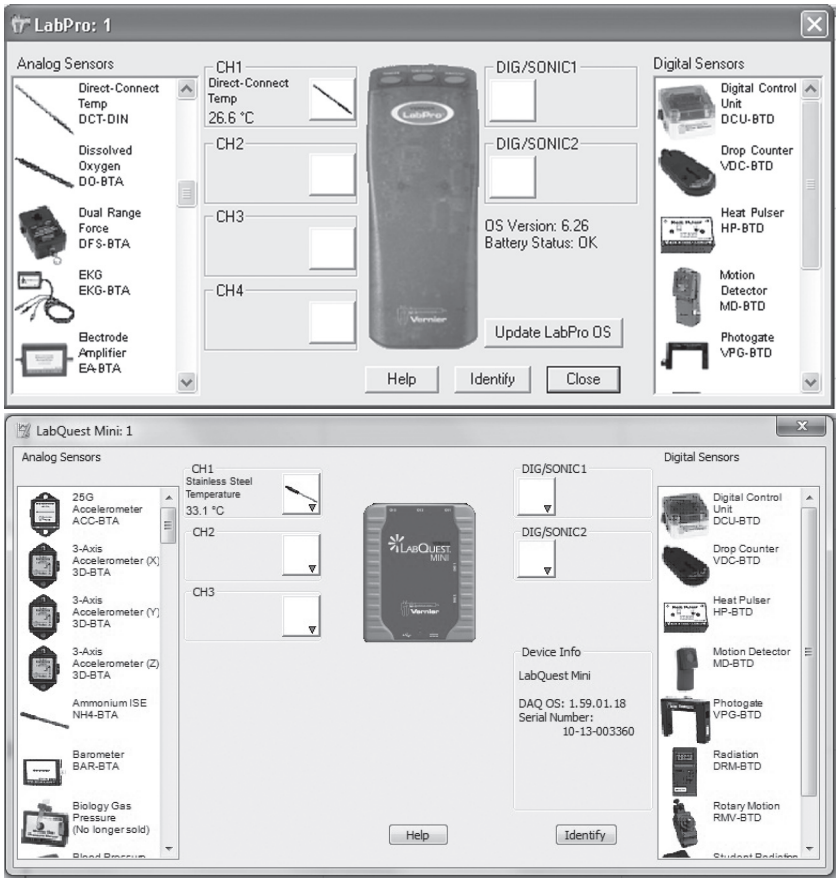


Figure R4.15 | Sensor Selection Window

III. Descriptions of Vernier sensors (probes)

A. Biology gas pressure sensor

1. Function

This sensor (Figure R4.16) measures the pressure of a gas. It can be used as a barometer, to measure volume, or to measure rates of chemical reactions that release or absorb gases.

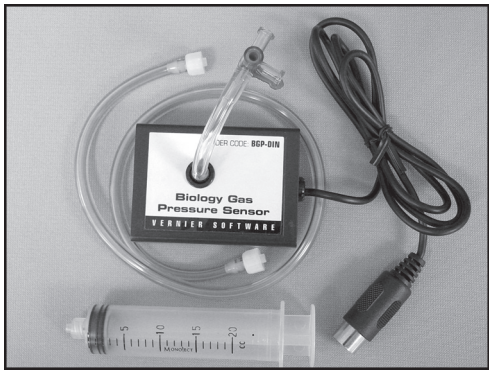


Figure R4.16 | Biology Gas Pressure Sensor

2. Mechanism

Inside the sensor is a compartment filled with a vacuum and sealed on one end by a flexible membrane. The pressure of the gas pushes the membrane and the force is measured. Because of its construction, **DO NOT LET IT GET WET**. A short section of tubing connects the sensor to a three-stem valve (Figure R4.17a-c). The direction of the lever on the valve points in the direction of the stem that is turned OFF and determines the path of the gas. One stem is attached to the tubing leading to the sample. The other stem allows you to release the pressure.

The units of measure for pressure are
1 atmosphere = 760 mm of Hg (mercury)
101.325 kilopascals
29.92 in. of Hg
1.013 bar
14.696 psi (pounds per square in.)

Figure R4.17a | Three-stem valve in OFF position—cuts off the flow of gas from the experimental sources.

Figure R4.17b | Three-stem valve in position to collect data—flow of gas from syringe is turned OFF.

Figure R4.17c | Three-stem valve in position to allow the syringe to remove air bubbles from tubing—flow to sensor is turned OFF.

Reference

3. Use

a. Turn the stem to the pressure sensor off (air will flow freely between the pressure release stem and the sample stem). Figure R4.18b.

b. Attach the tubing leading to the sample using *luer lock* adapter (these slip into the tube and screw onto the valve ends).

c. Allow conditions to stabilize.

d. Turn the blue valve on the three-way valve toward the syringe (air will flow freely between the pressure sensor stem and the sample stem). Figure R4.18b. **Always insure that sensor remains dry**.

B. Carbon dioxide (CO_2) sensor

1. Function

This sensor (Figure R4.18) measures the amount of carbon dioxide in air. It can be used to measure rates of combustion, chemical reactions that release CO_2, respiration, metabolic rate, or photosynthesis.

Figure R4.18 | CO_2 Gas Sensor

2. Mechanism

The sensor emits infrared radiation (IR) using a hot filament at one end of the tube. At the other end is a sensor that measures how much of the IR (4260 nm) passes through (similar to the way the spectrophotometer works). As the sensor's temperature increases it produces a higher voltage. The amount of radiation absorbed is proportional to the amount of CO_2 (think about global warming!).

The units of measure for CO_2 concentration are:

1 ppm (part per million)—1 part of gas (CO_2) by volume in 1 million volume units of the whole.

A concentration of 600 ppm for CO_2 would simply mean that there is 600 L of CO_2 gas per 1,000,000 L of air (or 0.6 ml of CO_2 per 1 L of air).

3. Use

a. Allow the sensor to heat up. This takes at least 90 seconds.

b. If you need to adjust the sampling rate, keep in mind that it takes at least 1 second for the sensor to update. Diffusion through the holes in the sensor can result in a 60 second delay before an accurate reading if there is a rapid, dramatic change in CO_2 concentration.

c. The CO_2 gas sensor has 2 settings: low range (0-10,000ppm) and high range (0-100,000ppm). If the value exceeds the maximum ppm (parts per million) for that setting during an experiment, you will not know by how much. Therefore you will need to exchange the air by opening the container.

d. Place the shaft of the CO_2 Gas Sensor in the opening of the container of air you are sampling by gently twisting the the sensor into the container opening.

e. Note that tilting the container while collecting data will result in fluctuating (and inaccurate) readings.

f. **DO NOT PLACE THE SENSOR INTO ANY LIQUID!** It is intended only for measuring *gaseous* CO_2 concentration.

C. Conductivity sensor

1. Function

Conductivity can be thought of as the ability to conduct an electric current. The ability of water to conduct electricity is increased by the number of charged chemical particles (ions) found in it. These ions are formed when certain substances are dissolved in water. They disassemble in a way that leaves one atom (or molecule) with an extra electron (– charge) and one atom an electron short (+ charge). For example NaCl ♦ Na^+ + Cl^-. The more ions, the greater conductivity. Conductivity can be used to determine whether water is hard (more ions) or soft (fewer ions). The saltier (saline), more acidic or more basic the water the greater its conductivity. Water treated with chlorine (Cl^-) will have greater conductivity. Runoff from fertilized fields will contain ammonium (NH_4^+), nitrate (NO_2^-), and phosphate (PO_4^+) and will have greater conductivity.

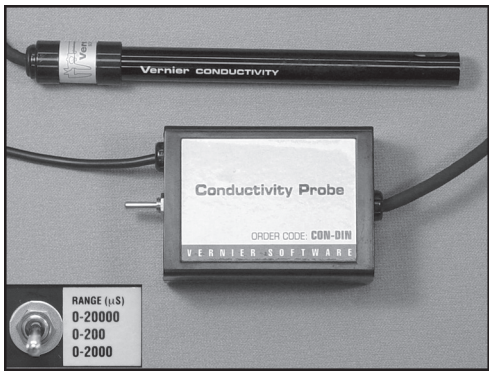


Figure R4.19 | Conductivity Sensor

2. Mechanism

The sensor (Figure R4.19) has two electrodes in it. By creating a potential difference (+ / -) between these, the sensor creates an electrical current (this represents the flow of charged atoms or ions) which flows between the electrodes. The sensor then converts current to voltage. The voltage is proportional to the number of ions or conductivity of the solution. **Because of the way it works, this sensor cannot be used in the same solution as the pH or D.O. probes while connected to the same LabPro interface.**

3. Use

a. Select the measuring range on the amplifier found between the sensor and the LabPro interface. (Ask your instructor for suggestions or use 0-2000S = 1000 mg/l).

b. Insert the sensor into the solution to be measured. Be sure the solution covers the hole near the end of the sensor completely.

c. Swirl gently or use a magnetic stirrer.

d. Wait until the sensor reading has stabilized.

e. **Before measuring another solution, rinse the sensor in distilled water. EVERY TIME YOU REMOVE THIS SENSOR FROM A SOLUTION *RINSE IT OFF WITH DISTILLED/ DEIONIZED WATER!!!***

f. When you are not using the probe, place it in a storage beaker which contains distilled water.

D. Dissolved oxygen sensor

1. Function

This probe measures the amount of oxygen present in water in mg/L. It could be used to measure oxygen released during photosynthesis, oxygen depletion during respiration, or Biochemical Oxygen Demand.

2. Mechanism

The sensor (Figure R4.20) allows oxygen to diffuse through a membrane and take part in chemical reactions that create an electrical current. **Do not let the sensor dry out. Do not touch the membrane** at the end. **DO NOT UNSCREW THE TIP OF THE PROBE!!!**

3. Use

a. If it has not been connected to a powered LabPro interface for 30 minutes, the sensor must be allowed to warm up for 30 minutes before use.

b. Insert the tip of the sensor into your sample. Gently place your sample on a stir plate set on **low speed**, so as not to mix oxygen into the sample. Remember that the sensor uses up the D.O. as it is measured. Remember too that turbulence adds oxygen to the water. Be sure no air bubbles contact the tip.

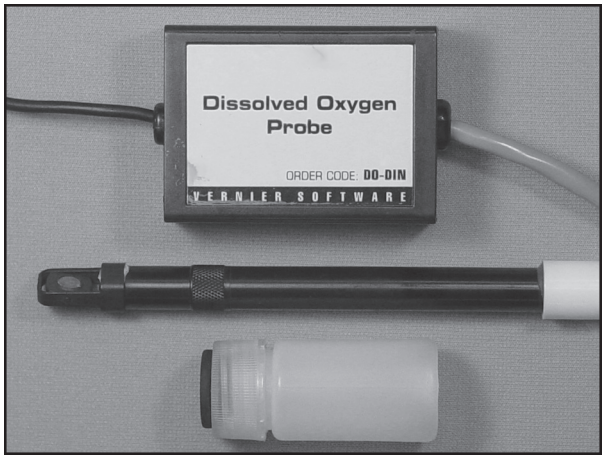


Figure R4.20 | Dissolved Oxygen Sensor

c. When the reading has stabilized, record your data.

d. Before measuring another solution, rinse the sensor in distillied water. **EVERY TIME YOU REMOVE THIS SENSOR FROM A SOLUTION *RINSE IT OFF WITH DISTILLED/ DEIONIZED WATER!!!***

e. When you are not using the probe, place it in a storage beaker which contains distilled water.

E. Light sensor

1. Function

This sensor (Figure R4.21) measures light intensity for visible light in lux. (No kidding!)

2. Mechanism

It contains a photocell. (Again—no kidding!)

3. Use

a. Select the appropriate range for the best sensitivity in low, indoor or outdoor conditions using the switch on the box between the sensor and the LabPro interface.

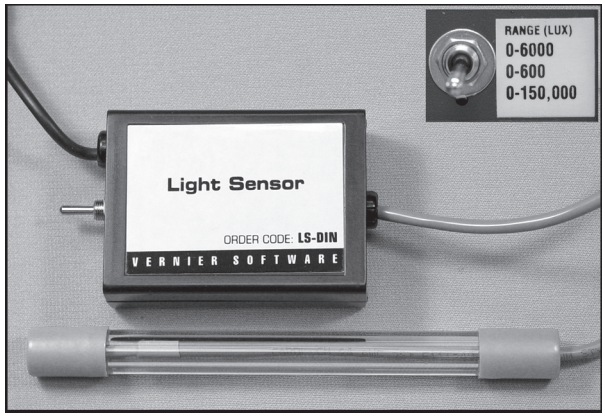


Figure R4.21 | Light Sensor

F. pH Sensor

1. Function

This sensor (Figure R4.22) measures how acidic or basic a solution is. The pH scale ranges from 0–14 with 7 being neutral (water). Values below 7 correspond to acids; values above 7 correspond to bases. Each unit is ten times more or less acidic than the adjacent unit.

2. Mechanism

The sensor consists of a gel-filled electrode that produces a voltage in proportion to the number of hydrogen ions present. The sensor needs no normal servicing but requires that it be stored upright in a special storage solution and not be allowed to dry out, or be stored in distilled water.

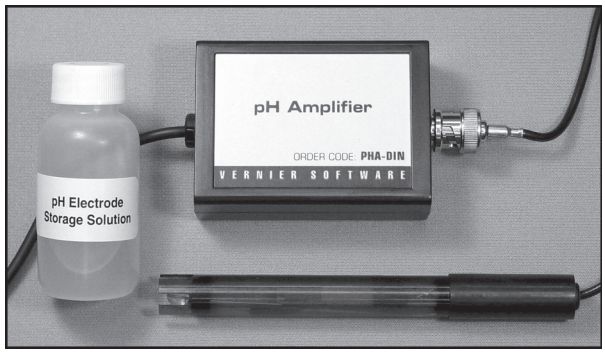


Figure R4.22 | pH Sensor

3. Use

a. Insert the sensor into a pH4 standard solution. Allow reading to stabilize. If the reading is close to 4, move on to the next step. If reading is not close to 4 then calibrate sensor using instructions provided with the sensor before moving on to the next step.

b. Insert the sensor into the sample solution so that it covers the tip and stir gently.

c. Take a reading when the pH appears stable (typically after about 30 seconds).

d. **When transferring between solutions, the pH sensor needs to be rinsed with distilled water. EVERY TIME YOU REMOVE THIS SENSOR FROM A SOLUTION *RINSE IT OFF WITH DEIONIZED WATER!!!***

e. When you are not using the probe, place it in a storage beaker which contains pH storage solution.

G. Relative humidity sensor

1. Function

This sensor (Figure R4.23) measures the concentration of water in the air expressed as a percentage of the total amount of water that the air can hold. This value can vary with temperature and pressure.

2. Mechanism

The sensor is housed inside a plastic box with slits (inset Figure R4.23). The sensor detects humidity in the air passing over it and generates a voltage. To work effectively, the air must be moving.

Figure R4.23 | Relative Humidity Sensor

3. Use

a. Place the sensor in the area of interest.

b. Create a current of air over the sensor using either a fan, if this does not interfere with the experiment, or by gently shaking the sensor.

H. Temperature sensor

1. Function

This sensor (Figure R4.24) measures the temperature of gases, fluids, or, in some cases, solids.

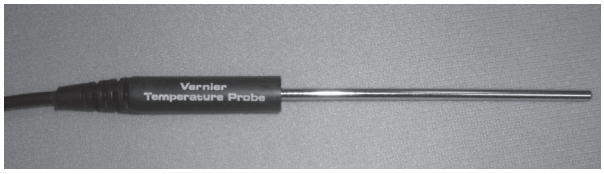


Figure R4.24 | Stainless Steel Temperature Sensor

2. Mechanism

This is a stainless steel temperature probe. Its operating range is -25ºC to 125ºC. The probe is constructed of a high-grade stainless steel providing a high level of corrosion resistance for use with a wide range of chemicals. This sensor will take 6-8 seconds to correctly register a change when immersed in water and about 30-60 seconds to register a change in air.

3. Use

a. Insert the tip of the sensor into the item to be measured. Do not submerge the probe beyond the stainless steel portion.

b. Wait for the temperature to stabilize and record.

c. Always wash the probe thoroughly after each use.

I. Oxygen sensor

1. Function

This sensor (Figure R4.25) measures the amount of oxygen in air. It can be used to measure the concentration of oxygen consumed or generated in a variety of chemical/biological processes, respiration, metabolic rate, or photosynthesis.

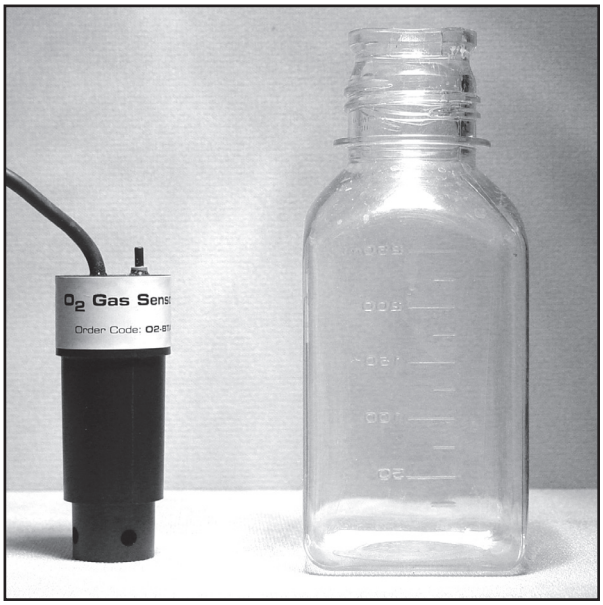


Figure R4.25 | O_2 Gas Sensor

2. Mechanism

The sensor measures the oxygen concentration in the range of 0 - 27% using an electrochemical cell. The cell contains a lead anode and a gold cathode immersed in an electrolyte. When oxygen molecules enter the cell, they get electrochemically reduced at the gold cathode. This electrochemical reaction generates a current that is proportional to the oxygen concentration between the electrodes. The current is measured across a resistance to generate a small voltage output.

The units of measure for O_2 concentration are:
Percent (%)
or
1ppm (part per million) – 1 part of gas (O_2) by volume in 1 million volume units of the whole.
or
1 ppt (part per thousand) — 1 part of gas (O_2) by volume in 1 thousand volume units of the whole.

3. Use

Allow the sensor to warm-up. This takes 1-2 minutes.

If you need to adjust the sampling rate, keep in mind that it takes at least 1 second for the sensor to update. Even though the sensor responds rather quickly to changes in O_2 concentration, remember that gas has to diffuse into the electrochemical cell located at the top of the sensor shaft before any changes in concentration can be detected. Since diffusion of gases is a fairly slow process, there can be some delay in readings. This is especially important to keep in mind when using this sensor with the CO_2- O_2 Tee.

The maximum value the sensor can detect accurately is 27%. If the value exceeds 27% during an experiment, you will not know by how much. There for you will need to exchange the air by opening the container. NOTE: The accepted value of atmospheric O_2 concentration is ~20.9%.

Place the shaft of the O_2 Gas Sensor in the opening of the container of air you are sampling by gently twisting the stopper on the shaft of the sensor into the container opening. **DO NOT TWIST THE SHAFT OF THE SENSOR** as you may damage it. **DO NOT PLACE THE SENSOR INTO ANY LIQUID!** It is intended only for measuring *gaseous* O_2 concentration.

Note that tilting the container while collecting data will result in fluctuating (and inaccurate) readings. **Very important: the O_2 gas sensor MUST remain upright at all times. Failure to do so can lead to malfunctions.**

IV. Guidelines for collecting data

A. Start program with an experiment file

Select  **> All Programs > Vernier Software > {template}** that you want to use. This starts the program with an experiment file containing pages with objects set up for use with specific probes. The files include:

1. Biology Gas Pressure Sensor
2. Biology Gas Pressure Sensor & CO_2
3. Biology Gas Pressure Sensor & Light
4. Biology Gas Pressure Sensor & Relative Humidity
5. Biology Gas Pressure Sensor & Temperature
6. CO_2
7. CO_2 & Temperature
8. Conductivity
9. Conductivity & Temperature
10. Dissolved Oxygen
11. Dissolved Oxygen & Temperature
12. Dissolved Oxygen & pH
13. Light
14. O_2
15. O_2 & CO_2
16. O_2 & Temperature
17. Relative Humidity
18. Temperature
19. Temperature & Temperature
20. Your own design*

B. Once you open the program, a **Page Information** window will appear. This will provide information about the probe(s) to be used, the current data collection settings, and other information that may be important. When you finish reading the message, click **OK**.

C. If a **Sensor Confirmation** window appears, verify that the probe you need is shown in the sensor box. You may then click **OK**.

D. Check your meter object to make sure that the data appears to be accurate. If the data looks weird (e.g., it's 150°C in the room!), check your probes, LabPro interface connection, and the program. If you still need assistance, alert your TA.

E. Decide if the sampling regime is correct

1. Will you be collecting data continuously (time-based) or only at certain points (event-based)?

2. Is the experimental duration long enough? You can always 

early.

3. Are the time units appropriate? (seconds, minutes, hours, etc.)

4. Are there too many or too few samples per time unit?

5. The default on our templates is set for time based data collection with various duration and sampling. When you open a file, the **Page Information** displayed will tell you the current data collection settings.

6. Any of the data collection settings may be changed through the **Data Collection** menu or  in the toolbar.

F. Collect data

1. Click 

when you want to begin the experiment.

2. Your data will be displayed in both the table and graph objects.

3. Wait until the end of the experimental run or click 

.

4. Change conditions if desired (auto scale, graph options, etc.)

5. You may examine, analyze, or print your data.

6. To collect an additional data set, **Store Latest Run** and click 

.

All of these functions are described in detail in the *Logger Pro* software section of this manual (see II.C within this section).

*This option is available so that you may create your own probe combinations. This also occurs when you select **New** in an existing file.

G. Analyze your data

1. In *Excel*

a. Copy all data in *Logger Pro* through **Edit > Select All** then **Edit > Copy** or click your mouse and drag it across the data you wish to copy then **Edit > Copy**.

b. Start a new *Excel* workbook or open a window to an existing workbook.*

c. Click on the desired cell and **Paste** data. Double-check that the correct data pasted into the *Excel* workbook. If not, in *Excel* go to **Edit > Office Clipboard**  and select **Clear All**. Return to *Logger Pro,* and try to copy and paste again.

2. In *Logger Pro*

a. Get descriptive statistics

i. Select a column of data or range of data on the graph.

ii. Select **Analyze**, then parameter of choice. For general statistics, you may also use  .

b. Fit a curve

i. Select a range of data on the graph.

ii. Go to **Analyze > Linear Fit** or **Curve Fit** to open the window (Figure R4.27). Once the window opens, select the type of curve you would like to apply to your data (e.g. linear, polynomial, power, etc.). Then click **Try Fit** and the line will be drawn on your graph.

iii. You may also select either **Linear Fit**  or **Curve Fit** from the toolbar to fit a line without a window.

iv. For more details and other options, see the section on the **Analyze** menu under Reference *Chemical and Physical Sensors*, II.C.

*For more information on the operation of *Excel*, go to Reference *Computer Basics*, II in this manual.

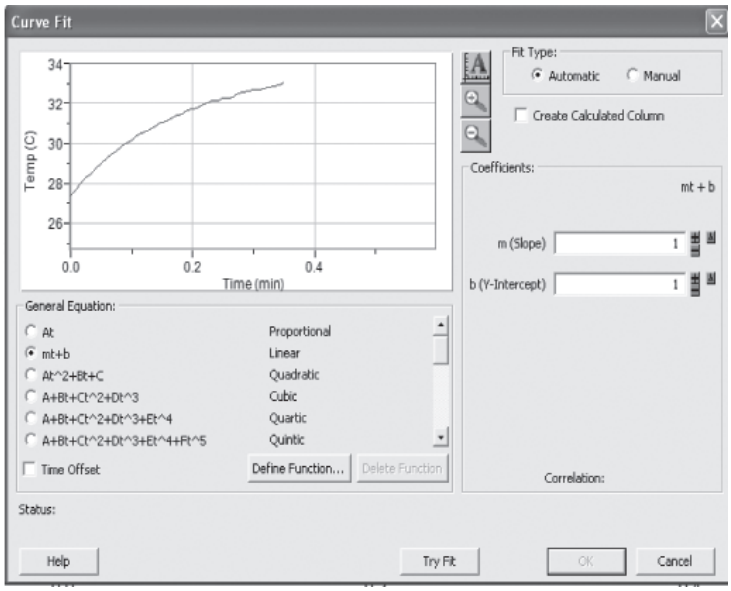


Figure R4.27 | Curve Fit Window.

Video Cameras

Written by Connie Russell and Moria Harmon

I. USB camera

The Logitech QuickCam USB camera (Figure R5.1) is a plug-and-play, auto-focus camera that allows you to record images illustrating your experimental methods such as experimental set-ups, people demonstrating procedures, etc. This section will describe the use of the USB camera itself and the use of the video-overlay/capture software necessary for viewing or storing images.

Figure R5.1 | Logitech USB camera

A. Plug the USB camera into an available USB port.

B. Open the Logitech camera software. 

C. Click  from the Logitech control panel (Figure R5.2).

D. Move the camera to show what you wish to take a picture of and click **Take Photo** (Figure R5.3).

Figure R5.3 | Capture Picture Screen For Logitech Camera

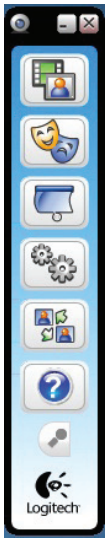


Figure R5.2 | Logitech Control Panel

Reference

E. Inside your MSWord document place your curser where you would like your image to appear in your report.

F. Click  in the Insert Tab (Figure R5.4).

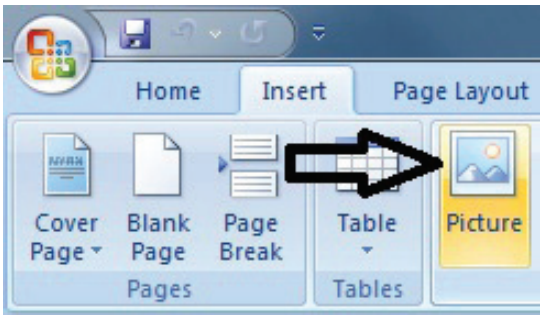


Figure R5.4 | *Word* Picture Insert Icon

II. Swiftcam

The SwiftCam is a specialized video camera that allows you to view objects on your computer screen. The SwiftCam's lens is mounted on a flexible arm attached to a sturdy base (Figure R5.5). This arrangement allows you to use the camera to view/record images of documents, of experimental set-ups, of dissections, of people's faces, from a microscope, etc. A microphone in the base allows you to record sound as well. This section will describe the use of the SwiftCam.

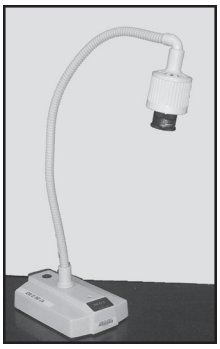


Figure R5.5 | SwiftCam Video-Camera

A. You can position the camera head any way you wish by simply bending the flexible arm into position.

B. The electrical power cord should be connected to the camera. Just depress the red power switch on the base until it lights.

C. Adjusting the lens

1. To adjust the focus, turn the focusing ring (collar – distal most portion of the lens).

2. To adjust the amount of light entering the lens, turn the iris adjustment ring (proximal portion of the lens). This manual diaphragm, although not as convenient as an automatic one, gives you complete control and allows you to use the camera with the Ultra-Lite fluorescent light on your microscope.

D. Switching the lens

1. If you wish to use the SwiftCam with a microscope, you will need to use the 16mm lens. This also will work for close-ups. If you want to use the SwiftCam to record or view any other macroscopic object, you will need to switch to an 8.5mm lens.

2. To remove the lens, grasp the lens between the focusing ring and the iris adjustment ring and turn counterclockwise to remove the lens. Do so firmly but gently to avoid breaking the lens' mount.

3. To replace the lens, carefully thread the lens into place and turn clockwise until the lens stops turning. Do so firmly but gently to avoid breaking the lens' mount.

E. Adjust the focus and lighting on the microscope and lens as needed.

1. If possible, select a microscope with an adjustable iris diaphragm rather than a disc diaphragm.

2. Slip the camera adapter (Figure R5.6) over the microscope eyepiece to form a cup and turn the thumbscrew to fix it into position.

3. Insert the SwiftCam lens into the camera adapter.

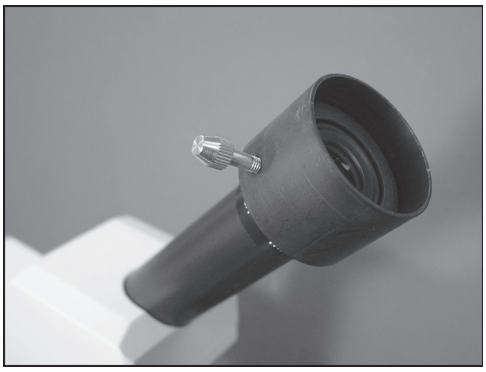


Figure R5.6 | Camera Adapter

4. Carefully adjust the flexible arm to force the lens snugly against the eyepiece (Figure R5.7).

5. Adjust the focus and lighting on the microscope and lens as needed.

6. When finished, your set-up should look something like that in Figure R5.8.

F. To view, capture, or print an image from a SwiftCam see section I.B-G.

Figure R5.7 | Force Lens Against the Eyepiece

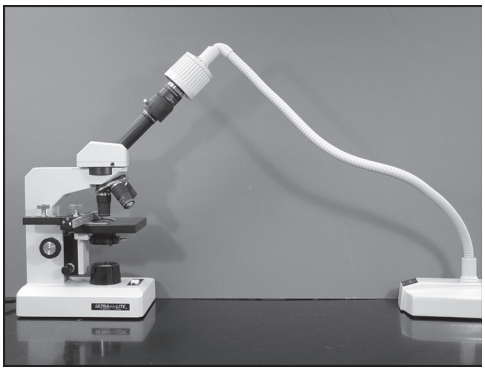


Figure R5.8 | SwiftCam Ready to Use

Using LOGAL Explorer

Written with help from Traci Weaver

Simulations are designed to illustrate the way some system works based on a mathematical model. is an example of simulation software. It allows the user the opportunity to control the value of some of the variables that enter the model. In this case, the software is specifically educational in nature and is designed for the introductory student. Therefore, it does not require that you understand any of the mathematics of the model (in fact it does not show you any of it). It provides you controls in the form of questions and pushbuttons, and it provides answers in the form of graphics.

To give you an idea what a model is and what a simulation does, why don't we investigate your savings account. Imagine that you have $1,000 to put in a bank that offers you 10% interest (OK, this sounds more like a fantasy than a simulation) and compounds yearly. How much money would you have next year?

Principle × Interest Rate = Interest & Interest + Principle = New balance

$1,000 * 0.1 = $100 & $100 + $1000 = $1,100

That should be easy. But what if I want to know when it will reach $1,000,000? What if I had $2,000 or I could get 15% interest, or only 5%? We could do these calculations by hand and list or plot the data, or we could develop a computer program that would let you enter the starting principle or balance and the interest rate and calculate the answers.

The simulations produced by LOGAL are more complex, but they have provided easy to understand outputs and controls. There are five simulations available: Cardiovascular system, Ecology, Genetics, Molecular Biology, and Photosynthesis. This set of instructions will explain the basic controls and summarize what simulations are available. There are much more extensive directions available from within the programs.

Occasionally, your laboratory or lecture instructor will point you in the direction of a particular simulation as a lecture, pre-lab or in-lab lesson or activity. However, or whenever you want to strengthen/test your understanding of a concept. Simulations count as experiments!!

I. Getting started

A. Click on **Start > Programs > LOGAL Biology**.

B. Choose the general simulation (Cardiovascular System, Ecology, Genetics, Molecular Biology, or Photosynthesis).

C. When you get the LOGAL Explorer 3.04 "Splash Screen," click on it to continue (Figure R6.1).

D. You are at the main menu (Figure R6.2) and can now choose to:

1. Get an overview of the way the program and the specific topic investigation work by clicking **First Look**.
2. Perform some basic, introductory, investigations by clicking **Explorations**.
3. Perform some intermediate level activities involving somewhat more complex investigations by clicking **Core Inquiries**.
4. Perform lengthy investigations of more open-ended questions that involve integrating knowledge and designing your own meth odology by clicking **Independent Investigations**.
5. If you want to learn more about how the lessons are programmed click **Workshops**.
6. If you need help learning about how to use the software click **On-screen Reference**.
7. If you want to exit, click **Quit**.

Figure R6.1 | LOGAL Splash Screen

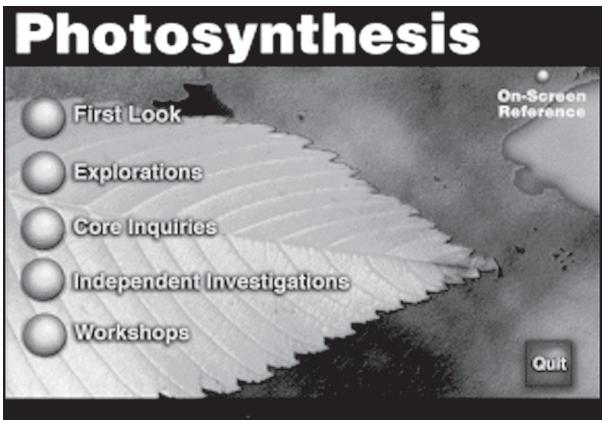


Figure R6.2 | Photosynthesis Main Menu

II. Simulation screen components

A. Menu bar

Except for HELP, leave this alone!! You can alter the parameters so the simulation won't work.

B. Work window

This window (Figure R6.3) is the majority of the screen. In it you will find instructions, questions and places for you to type answers, hypertext (blue), and buttons to click for input, graphs, and digital displays for output.

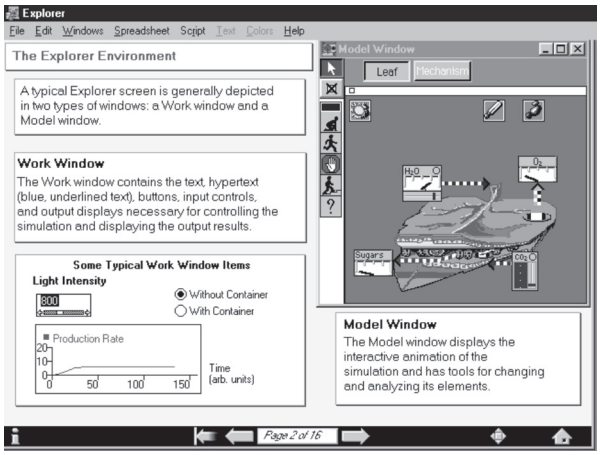


Figure R6.3 | Typical Simulation Screen

C. Model window

This is where the animation appears along with the basic tools for its control and the topic specific tools for control. The basic tools include:

1. Pointer/Hand tool

This tool lets you click and adjust controls made available by the simulation.

2. Reset

Clicking this tool resets the simulation to its initial values.

3. Run

Starts the simulation.

4. Stop

Clicking this tool immediately halts the simulation. While stopped you can reset to the initial values or change them.

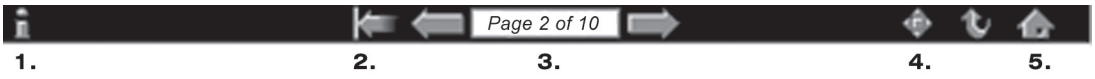
5. Single Step

This tool lets you run through the simulation one "step" each time you click it. This will give you more time to watch rapidly paced actions or rates of reactions. It also makes it easier to set the simulation to a particular point and to record data at specific points.

6. Help

Clicking this tool gets help on specific topics.

D. Navigation panel



This is the black bar at the bottom of the screen. It provides for movement to various screens within the lesson and a button that permits you to go back to the main menu. These controls include:

1. The Information Window
This will open up a help window describing the Navigation panel controls and the buttons that control audio and video clips.

2. The Restart Button
This restarts the activity or lesson.

3. The Paging Buttons
These arrows allow you to move forward or backward to the next "page" in the lesson.

4. The Content Window
This window will show you the contents of the lesson in a flow chart form and allow you to jump to the pages in which you are interested.

5. The Home Button
This will return you to the main menu.

E. LOGAL biology programs

The following list outlines all of the pre-programmed lessons. You may use them to extend your investigation or to review for lecture. You can also conduct simulated experiments of your own design by manipulating the controls in the **Model Window** as described in the or sections. Data collected via simulation can be used as "experimental" data, i.e. these lessons count as experiments.

1. Cardiovascular systems simulation

Area	Explorations (Basic)	Core Inquiries (Intermediate)	Ind. Investigation (Advanced)
Overview	Introduction		
Cardiac anatomy	Blood circulation		
Cardiac Concepts: Heart rate, blood flow & pressure Stroke volume	A check-up for Mr. Hartman A closer look at the human pump	Controlling blood flow Blood flow rate Blood supply Blood pressure Controlling blood pressure A pressure control mechanism	Blood distribution
Fitness & disorders	Fitness & the heart	Heart attack! Heart problems	Hardened arteries Cardiac disorder

Table R6.1 | Cardiovascular Concepts and Relevant Lessons

2. Population ecology simulation

Area	Explorations (Basic)	Core Inquiries (Intermediate)	Ind. Investigation (Advanced)
Overview	Introduction		
Adaptations		Eating efficiently Survival strategies Environmental changes	Global warming
Population growth	Observing population growth Modifying population growth	S & J curves Carrying capacity of grass	Design an animal
Predation & competition	Two populations: What's the difference? Two competing populations Enter predator	Competition Changing environmental conditions Predation Competition pressure	
System management	Maintaining a balance	Introducing a virus Effect of food supply Competition pressure Maintaining system balance	Design an ecosystem

Table R6.2 | Ecology Concepts and Relevant Lessons

3. Genetics simulation

Area	Explorations	Core Inquiries	Ind. Investigation
Monohybrid inheritance (Mendel's 1st Law)	Mendelism Testing Mendel		Mystery individuals Genetic counseling
Dihybrid inheritance (Mendel's 2nd Law)		Dihybrid crosses	Mystery individuals
Sex linkage		Sex linkage	Mystery individuals
Extensions to mendelism		Variations to dominance Three's a crowd Fatal flaw	Mystery individuals Ordering three genes
Meiosis		Meiosis	
Linkage & genetic mapping		Linkage & variation	Mapping two genes Ordering three genes
Population genetics & environmental effects		Population genetics I Population genetics II	Catastrophe Environmental effects
Basic probability	Genetic probability		

Table R6.3 | Genetics Concepts and Relevant Lessons

4. Molecular biology simulation

Area	Explorations	Core Inquiries	Ind. Investigation
General molecular biology	Who's the boss? The genetic material	Interrupted genes Natural mutations Recombinant DNA	
Genetic engineering	Mapping DNA Genetic engineering	Interrupted genes Recombinant DNA The human genome database	Designer genes
Mutations		Natural mutations	Chemical mutations
Replication	DNA replication		
Transcription	RNA: an intermediary	Interrupted genes	From protein to DNA
Translation	Protein production	Codons & frames The genetic code Translation stops	From protein to DNA Breaking the genetic code

Table R6.4 | Molecular Genetics Concepts and Relevant Lessons

5. Photosynthesis simulation

Area	Explorations (Basic)	Core Inquiries (Intermediate)	Ind. Investigation (Advanced)
Overview	Introduction		
Calvin-Benson cycle		Light & photosynthesis Photosynthetic reactions Energy storage Carbon dioxide fixation Energy in the Calvin-Benson cycle	
Environmental factors	What is photosynthesis? Light, carbon dioxide, water, temperature, humidity: What affects photosynthesis rates the most? Why are leaves green?	Limiting factors I Limiting factors II	Sun & shade plants The origin of fossil fuels
Light Reactions		Reactants & products Photosynthetic reactions Energy storage The electrons The proteins & oxygen	
Respiration	Respiration vs. photosynthesis		
Stomates			The delicate balance

Table R6.5 | Photosynthesis Concepts and Relevant Lessons

How to Measure

Written by Connie Russell

I. Linear dimensions

Measuring linear dimensions (the distance between two points) is usually associated with using a ruler or a tape measure. For measuring objects in lab, you want to make as precise a measurement as is possible. One way of making more accurate measurements is to use calipers (Figure R7.1).

Calipers are often used to measure three-dimensional objects like the width of a turtle shell, but they can also be used to measure two-dimensional distances. The calipers in the lab are dial calipers. They have both metric and English scales and are accurate to the millimeter or 0.001 inches. To measure an object:

A. Make sure that the calipers are zeroed. That is, make sure that the pointer on the dial is pointed exactly at 0 (**A** in Figure R7.1). If necessary, you can calibrate the dial by gently using the ridges on the clear plastic face to turn the dial until the pointer and the tick mark at 0 line up exactly, when the jaws are shut tight.

B. To measure outer dimensions of an object, use your thumb to roll the wheel (**B** in Figure R7.1) counterclockwise until the object will fit into the area between the lower set of jaws (**C** in Figure R7.1). This provides accurate measurement of any object, such as a hissing cockroach, that will fit between the set of jaws.

C. These calipers are also capable of measuring the inner dimensions of objects such as pipes, cans, etc. For these types of measurements, the upper set of jaws is placed inside the object to be measured and the wheel is turned until the set of jaws is firmly seated in the space to be measured (Figure R7.2).

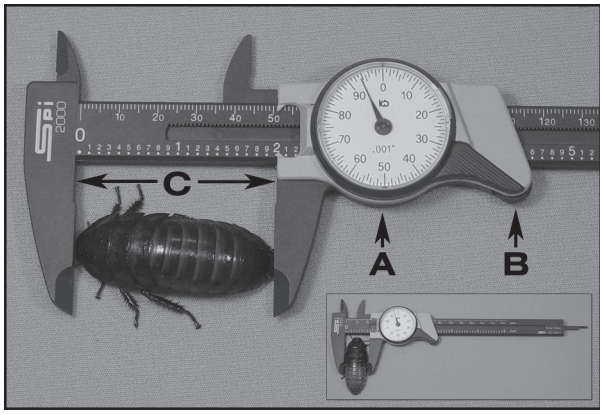


Figure R7.1 | Dial Calipers

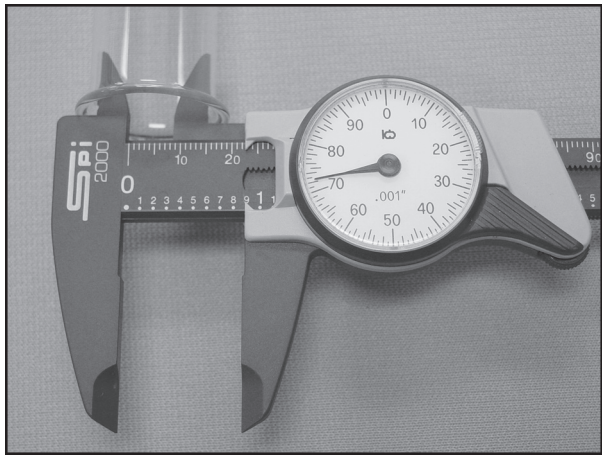


Figure R7.2 | Measuring the Inner Diameter of a Test Tube

Reference

D. You can also measure depth with calipers. You do this by aligning the metal rod found at the end of the calipers opposite from the jaws with the dimension on the object to be measured (Figure R7.3). As the wheel is turned, the rod extends and its length is reported on the dial.

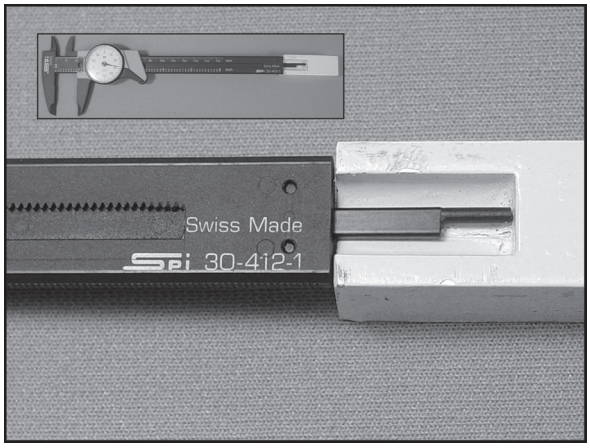


Figure R7.3 | Depth Measurement

II. Surface area

A. Regularly-shaped objects

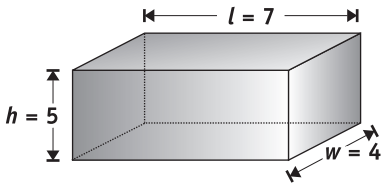
The following formulas and figures can be used to calculate the surface area (S) of regular geometric shapes.

1. Rectangular box

S = 2(lw + wh + lh) where l is the length, w is the width, and h is the height.

Example:

S = 2{(7)(4) + (4)(5) + (7)(5)}
= 2(28 + 20 + 35)
= 2(83) = 166



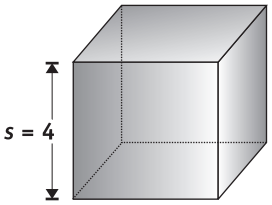
If the unit of measure in this example is inches, then the box has a surface area of 166 in^2.

2. Cube

S = 6s^2 where s is the side.

Example:

S = 6(4^2)
= 6(16)
= 96



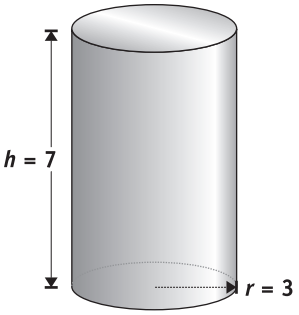
If the unit of measure in this example is inches, then the box has a surface area of 96 in^2.

3. Cylinder

S = 2πrh + 2πr^2 where r is the radius and h is the height.

Example:

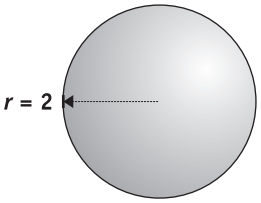
S = 2 (3.14)(3)(7) + (2)(3.14)(3^2)
= 131.88 + 56.52
= 188.4



If the unit of measure in this example is feet, then the cylinder has a surface area of 188.4 ft^2.

4. Sphere

$S = 4\pi r^2$ where r is the radius.



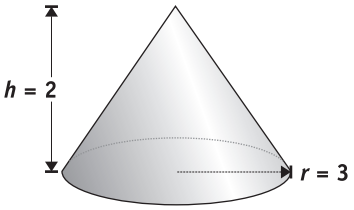
Example:

$$S = 4\ (3.14)\ (2^2)$$
$$= 4\ (3.14)\ (4)$$
$$= 50.24$$

If the unit of measure in this example is in kilometers, then the surface area of the sphere is 50.24 km^2.

5. Cone

$S = \pi r^2 + \pi r \sqrt{r^2 + h^2}$ where r is the radius and h is the height.



Example:

$$S = (3.14)(3^2) + (3.14)(3)\ \sqrt{3^2 + 2^2}$$
$$= (3.14)(9) + (9.42)(3.61)$$
$$= 28.26 + 34.01$$
$$= 62.27$$

If the unit of measure in this example is in millimeters, then the surface area of the cone is 62.27 mm^2.

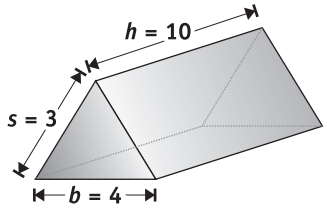
6. Triangular prism

$S = Ph + 2B$ where P is the base perimeter (the length of all sides of the triangle added together) and h is the height of the prism and B is the area of the base ($B = \frac{1}{2}bs$ where b is the length and s is the side of the base of the triangle.)

Example:

$P = 3 + 3 + 4$
$= 10$

$B = \frac{1}{2}\ (4)(3)$
$= \frac{1}{2}\ (12)$
$= 6$



$S = (10)(10) + 2(6)$
$= 100 + 12$
$= 112$

If the unit of measure in this example is in yards, then the surface area of the prism is 112 yds^2.

B. Irregularly-shaped objects

The surface area of irregularly-shaped objects (such as a leaf or an animal's torso) can be estimated by:

1. Dividing the irregular shape into regular shapes, calculating the surface area of the regular shapes (minus any non-surface faces), then adding the surface areas of each shape to give a total surface area.
2. Cutting a square of the object of a unit—say 1 cm^2 and weighing that object. Next, take the entire object and weigh it. Finally, divide the weight of the entire object by the weight of the unit.

   Note: for this to work, the object must be either two-dimensional or of a constant thickness. You can trace some objects onto paper.

III. Measuring Mass (weight)

In these labs, you will have electronic, two-button balances (Figure R7.4) with a capacity of 150g and an accuracy of 0.01g. They may be used to weigh any solid or powdered object, but should not be used to weigh liquids.

To weigh an object:

A. Place the balance on a flat surface. Make sure that it is plugged in.

B. Push the Tare/On button once, then wait until the LCD panel reads "**0.00 g**."

C. If the object to be weighed is a solid object (such as a block of wood), it may be placed directly on the balance.

D. If the object is a powdered substance that you are weighing to put into a solu-tion (such as agarose), obtain a weigh-ing dish big enough to hold your entire sample and place it EMPTY on the balance. When the mass of the weighing dish has stabilized, push the Tare/On button again. This will reset the balance to read "**0.00 g**." This is what is meant by tare. You may now weigh your powder.

Figure R7.4 | Cynmar Electronic Balance

E. If you place an object that is too heavy on the balance the LCD panel will read "**- - - - -**." Remove the object and try dividing it into pieces if possible. Add the mass of the pieces together to get a total mass. If you can't divide the object, request a balance with a higher mass capacity from the TA.

F. To turn the balance off, hold down the OFF button until the LCD panel is blank.

IV. Measuring volumes

A. Using a pipette (liquid)

1. Select the smallest pipette that will hold the volume you need. Be sure you know to what measure the gradations correspond (1.0 ml, 0.2 ml, 0.1 ml, etc.).
2. Depress the plunger on the Pipette Pump™ and insert the pipette (Figure R7.5) into the liquid to be measured.
3. Rotate the wheel toward the pipette to draw fluid up until the desired amount is withdrawn.
4. To dispense volume quickly—depress lever until liquid no longer flows. Do NOT shake to dispense remaining liquid.
5. To dispense volume slowly—rotate the wheel away from the pipette until the desired amount is dispensed.

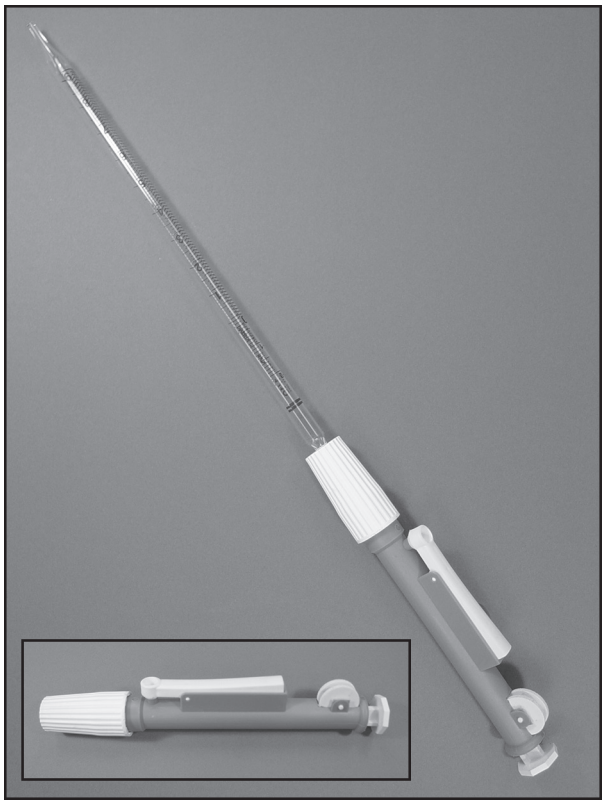


Figure R7.5 | Pipette Pump

B. Using a micropipette (liquid)

1. The micropipettes (a.k.a. pipettors) you are using have units of µl (microliters—10^{-6} liters). Using the dial on the top of the plunger (Figure R7.6), select the volume you wish to deliver. **These micropipettors cannot measure less than 5µl or more than 50 µL. Do NOT set them beyond these points.**
2. Seat a tip from the tip box firmly on the end of the pipettor by gently but firmly pressing the pipettor end into the top of the tip. **Do not use the micropipette without a tip!**

   Note: if you touch the end of the tip to anything once you have removed it from the sterile tip container, it will no longer be sterile.
3. Depress the plunger on the end of the pipettor with the thumb of your dominant hand until you feel a "stop." You will still be able to depress the plunger further, but resist the urge to do so.

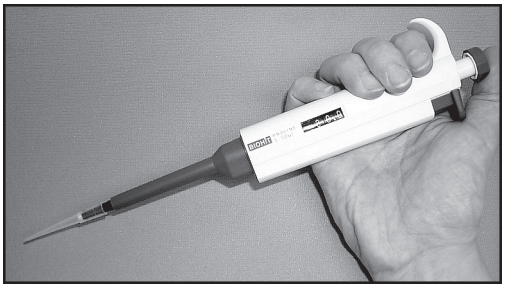


Figure R7.6 | Biohit Micropipettor Used in Lab

Reference

4. Place the pipettor tip into the liquid to be dispensed. Slowly allow the plunger to return to its starting position. The pipettor is now loaded to deliver the desired volume.
5. Place the pipettor tip into the microtube, well in an electrophoresis gel, or other container in which you wish to deliver the liquid.
6. UNLESS YOU ARE LOADING A GEL, depress the plunger to the second "stop." If you are loading a gel, going to the second stop may cause you to introduce an air bubble to the well or may cause you to "blow" your DNA or protein sample into the gel buffer. WHEN LOADING AN ELECTROPHORESIS GEL, depress the plunger to the first "stop" only.
7. Once the liquid is dispensed, eject the pipette tip into the appropriate container by pressing the tip ejector. If dispensing a bacterial sample make sure to eject the tip into a BIOHAZARD bag.

C. Using a graduated cylinder (liquid)

1. Select the smallest graduated cylinder that will hold the volume you need. Be sure you know to what measure the gradations correspond (1.0 ml, 0.2 ml, 0.1 ml, etc.).
2. Be sure the graduated cylinder is on a flat surface and that you view the value at eye-level. If plastic—read the value straight across.
3. If glass—read the value at the bottom of the meniscus (the water line on Figure R7.7 indicated by the arrow)—the concave surface at the top of the liquid. For example, the volume measured by this graduated cylinder would be 20 ml.

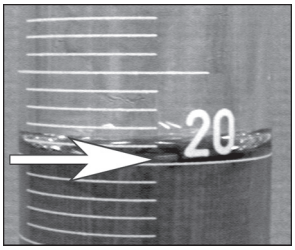


Figure R7.7 | Graduated Cylinder Meniscus

D. Using displacement (solid)

1. Fill a graduated beaker or cylinder with a known quantity of water and record the volume.
2. Place the object to be measured in the water and be sure it is completely submerged (repeat step 1 if needed).
3. Record the new (total) volume, then subtract the starting volume to obtain the object's volume.

E. Using weight (solid or liquid)

1. Start with an object of the same material and of known volume as obtained by another method.
2. Weigh the object of known volume and divide the weight by the volume to get a weight per unit volume.
3. Now weigh your object of unknown volume and divide its weight by that of the weight per unit volume calculated in step 2 to obtain its volume.

F. Using linear dimensions (solid)

1. Obtain an object with regular dimensions (sphere, cube, pyramid, cylinder).

2. Measure its dimensions (length and width [or diameter] and height) as described in II.A.

3. Apply one of the formulas in Table R7.1 below for volume.

Shape	Volume
Rectangular box	length × width × height
Cylinder	$\pi \times (\text{diameter}/2)^2 \times \text{height}$
Right regular pyramid	$^1/_3 \times$ width of base × length of base × height
Right circular cone	$^1/_3 \times \pi \times (\text{diameter}/2)^2 \times \text{height}$
Sphere	$^4/_3 \times \pi \times (\text{diameter}/2)^3$
Ring	$2 \times \pi^2 \times (\text{diameter of cross section}/2)^2 \times (\text{diameter of ring}/2)$

Table R7.1 | Formulas for Calculating Volume Using Linear Dimensions

G. Using pressure (gas or liquid)

This method lets you measure changes in volume by taking advantage of the following relationship among pressure, volume, and temperature:

$$\frac{P_1V_1}{T_1} = \frac{P_2V_2}{T_2}$$

1. Start by measuring a liquid volume (using one of the methods described above) and record the volume measured.

2. Place the measured liquid in a container or tube that can be sealed and attached to a device for measuring pressure (see Reference *Chemical and Physical Sensors*, III.A).

3. Determine the starting pressure (see Reference *Chemical and Physical Sensors*, III.A).

4. Change the volume (this will be done as part of some experiment).

5. Record the pressure.

6. If temperature is a constant then:

$$V_2 = \frac{P_1V_1}{P_2}$$

V. Preparing Solutions

A. Percent Solutions

In biology, most general solutions are simple percent solutions. Other solutions based on the chemical properties of the solute may also be used, such as molarity, or osmolarity, however, a percent solution serves a very useful tool for most applications.

Preparing a percent solution is based on the 100 ml volume. One starts by making a 100 ml volume of an easily prepared, concentrated solution. So to make a 0.9% NaCl (salt) solution the easiest way to start is by creating a 10% W/V (weight/volume) stock solution. To do so, weigh out 10 grams of sodium chloride (salt) and then add this to100 ml of deionized water. (To create other percentages, adjust the amount of solute; the volume of solvent is always 100 ml.)

Unlike the stock solution, to prepare dilutions of a stock, you must remember you are adding one solution to another and must account for the solvent. When making 300 ml our 0.9% salt solution, you know the concentration of our stock solution (10%), the final concentration of our solution (0.9%) and the final volume we need to prepare (300 ml). Armed with this information, we can determine the amount of our stock solution required to make the 0.9% saline as follows:

Stock volume needed = $\frac{\text{final concentration} * \text{final volume}}{\text{Stock concentration}}$

So for our example: $V_1 = \frac{0.9 * 300}{10}$

$V_1 = 27$ ml

To account for the solvent in the stock solution, you subtract the volume of stock solution from the final volume. Thus, to prepare our solution, you combine 27 ml of 10% stock solution and 273 ml of deionized water to equal 300 ml of a 0.9% NaCl solution. The easiest way to do this is to measure out the stock solution in a graduated cylinder, then add enough deionized water to reach the final volume.

B. Serial Dilution

Serial dilution is a quick way to make a set of solutions of equally-spaced, decreasing concentrations, e.g. each solution might be 1/10 the concentration of the previous one. This is useful for testing the effect of concentration on some dependent variable. The general procedure is to start with a stock solution (highest concentration possible) or initial dilution (highest concentration you want to use), then transfer a specific small amount of solution to an empty container and add enough solvent to reach the original or desired volume. The resulting solution is then used to make the next one.

Example: Put 10 ml of a 10% solution in a beaker and add 90 ml of solvent to make 100ml of a 1% solution. This is a 1:10 dilution. To make a 1:100 dilution, take 10 ml of the 1% solution, put that in a clean beaker and add 90ml of water to make 100ml of a .1% solution. The Result: 10% (stock), 1%, and 0.1% or 1, 1:10, 1:100

So to make a desired amount of a particular dilution, you should multiply the amount you need by the dilution and pour that amount of the starting solution into a clean beaker then add enough solvent to reach the desired volume.

Example: You want to make four solutions in a 1:4 series of exactly 300 ml each starting with just enough stock solution to make the first solution 1:4 of stock. For the last solution to be 300ml, you need 0.25*300 or 75ml of the previous. To be left with 300ml of the previous, you need 375ml to start. So take 0.25*375=93.25ml of the prior and add enough water to make 375ml of your previous dilution and so on. The following table shows you what you do:

From the stock you take	99.61ml	of stock and add	298.83ml of solvent	to get 398.44ml of 1:4
then you take	98.44 ml	of 1:4 and add	295.31ml of solvent	to get 393.75 ml of 1:16
then you take	281.25 ml	of 1:16 and add	93.75 ml of solvent	to get 375 ml of 1:64
finally you take	75ml	of 1:64 and add	225 ml of solvent	to get 300 ml of 1:256

If it is OK to have a little waste, then it is faster and easier to estimate:

Take 100ml of stock and 300 ml of solvent = 400 ml of 1:4 (you will be left with 300ml)

Take 100ml of 1:4 and 300ml of solvent = 400ml of 1:16 (you will be left with 300ml)

Take 100ml of 1:4 and 300ml of solvent = 400ml of 1:64 (you will be left with 300ml)

Take 100ml of 1:4 and 300ml of solvent = 400ml of 1:256 (you have more than you need – so pour it off.)

Statistical Tests

I. Rationale

Although most uses of statistics are considered beyond what we expect of you, you might want to take the opportunity to learn to apply them. Learning about statistics will help you understand why many statements or reports made to the public are considered misleading and why scientists assign probabilities and not absolutes when they testify in court. Correctly applying statistical tests to your data will impress your reader (lab instructor) and earn you additional credit.

When scientists publish the results of their work in a scientific article, statistics are essential. Once a scientific paper is sent out for publication it is reviewed by a panel of scientists who do similar research. This process is called peer review. Typically, without statistics, the reviewers will not believe the interpretations of the data and will reject the article and the research. Why? Well in general, probabilities generated by statistical tests indicate whether the events that transpired are a matter of chance or not. How likely is it that you could have gotten the results you did, not because the experimental variable has an effect, but because of a chance occurrence?

On what are statistical tests based? Probabilities, and here is how they are used. Imagine you and a friend are in a race and she wins. Is she truly faster than you or was it just her lucky day? Well if all things were equal, you would tie every time. But there are always slight differences in the track, the wind, etc., so it is more likely that you might win one race and she the next. On average, you win one race out of two, or ½ the time, and she wins one race out of two, or she wins ½ the time. But if she were to win twice in a row, does this mean she is really faster? Well, for each race, the chance you win is ½ and the chance that she wins is ½. The odds of her winning two in a row are ½ * ½ =¼= .25 (= one chance in four). This is still pretty high. What about 8 times in a row? Well it's ½ * ½ * ½ * ½ * ½ * ½ * ½ * ½ or 0.00390625. So this might occur by chance only in approximately 4 cases out of 1000. You might then conclude that the hypothesis that there is no difference between your running speed and hers is unlikely to be true. You would reject that hypothesis and suggest that these data support the hypothesis that she really is faster. Does that mean she is, for sure? Have you proven that she is faster than you? No, but the chances that you are as fast or faster than she is are quite small.

Although the methods are different, this is how statistical tests are used. Based on certain mathematical assumptions, one can determine how likely it is that the results occurred by chance rather than due to some factor or event. The convention is that if the probability (p) is less than five chances in one hundred ($p < .05$), the results are considered different from chance. The term significantly is reserved for this use. It does not mean important or meaningful or any similar term and it should not be used in any other way.

There are many different statistical tests that can be used for testing all sorts of relationships. You are welcome to research and use whichever is appropriate. This module includes five tests, all of which are included in the software.

t-test—This procedure tests whether the average of one group is different than that of another.

Paired t-test—This procedure tests whether the average of a group of individuals changes after something has been done to them. This is a before and after test.

One-Way ANOVA—This procedure tests whether the averages of three or more groups are different from each other.

Correlation/Regression—Do the values of one variable increase or decrease as do those of another variable? For example, it could be used to test data collected to determine whether grades increase as study time increases.

Chi-squared (χ^2)—This procedure tests whether a frequency distribution is different from an expected one or whether the distribution that occurs under one circumstance is the same as the distribution under another circumstance. For example, it could be used to determine whether the number of A's, B's, C's, etc. in a class taught by professor X are different from those in professor Y's class.

II. t-Test

A. Purpose

This procedure tests whether the means (averages) of two groups are equal. For example, let's imagine you want to know whether diet affects the rate at which mice run. You could race pairs of mice, count the number of races won by those eating Diet A and those eating Diet B and then compare the counts via χ^2 (see below). But that doesn't take absolute speed into consideration, just the relative speed of each pair. Instead you could measure the running speed of several (maybe 10) mice that eat Diet A and a similar number eating Diet B. Then you could average the speeds for each group and compare those. Although the average is a good single number to use to describe a characteristic of a group, it omits the characteristic of , i.e., how much individuals vary from the mean. For this we need a measure of variability, the variance. The t-test compares means while taking variance into consideration.

B. Requirements

1. The dependent variable must be continuous, i.e. measured not counted. (An example of a measured variable would be rate of water consumption in a group of camels, while an exam ple of a counted variable might be 14 chickens crossed the road.)
2. The same individual is not assigned to both groups (see paired t-test).
3. The dependent variable must be normally distributed (think of a bell-shaped grading curve where you have lots of students with grades in the C (average) range and fewer students in the above or below average ranges). We will not worry about this, but we really should.
4. The selection of the appropriate t-test formula (either t-test for equal variances or t-test for unequal variances) is based on whether or not the data are from two groups with equal vari ances. This can be determined with an F-test.

C. Procedure

1. Open Excel.
2. Enter your data into a table consisting of two columns with headings similar to Table R8.1.
3. To determine which t-test to use you must start by testing if the variances of the two groups are equal by selecting the **Data** tab then clicking on the **Data Analysis** icon.
4. Choose **F-Test Two Sample for Variances** from the dialog box. Click **OK**.

Diet A	Diet B
2	5
3	4
3.5	6
4	5
2	4
3	2

Table R8.1 | Sample Data for t-Test

5. Select the data for Variable 1 (Figure R8.1) by clicking on the little red arrow on that row of the dialog box, then selecting the cells, including the heading, for one column of data. Then press **Enter**.

6. Select the data for Variable 2 by clicking on the little red arrow on that row of the dialog box, then selecting the cells, including the heading, for the other column of data. Then press **Enter**.

7. Click in the **Labels** box to identify the first row of each set of data as the label.

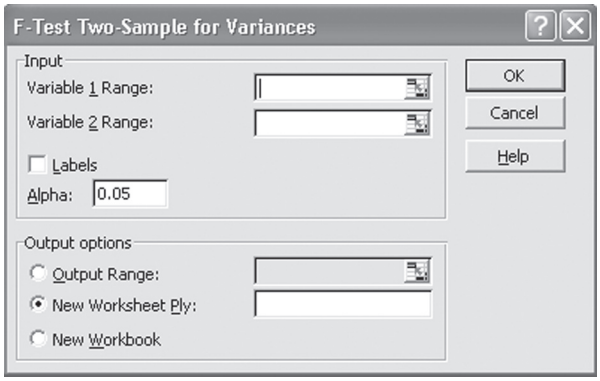


Figure R8.1 | F-Test Window

8. Change the alpha level to .025 (This sets the level of acceptance/rejection to one in twenty (5%) for this test. Consult one of the statistics books in the lab or LRC for an explanation.).

9. Click the circle (radio button) next to **Output Range**, then the little red arrow on that row of the dialog box, finally select a cell in which to place the results (there should be two empty columns to the right and nine empty rows below the cell selected).

10. Click **OK** when you are ready and will produce results as seen in Table R8.2.

ROW	D	C	E
1	F-Test Two-Sample for Variances		
2			
3		*Diet B*	*Diet A*
4	Mean	4.333333	2.916667
5	Variance	1.866667	0.641667
6	Observations	6	6
7	df	5	5
8	F	2.909090	
9	P(F ≤ f) one-tail	0.13307	
10	F Critical one-tail	7.146354	

Table R8.2 | Results of F-Test on Sample Data for t-Test

11. Look at the row in the table labeled **Variance** (row 5). If the left column contains a value that is lower than the right, repeat steps 4-9, reversing the column you select as Variable 1 and Variable 2.

12. Compare the Value for F to the critical value (row 10). If the Value for F is larger, then the two variances are unequal, and you must choose the t-test assuming unequal variances. If it is smaller, then you can assume that the variances are equal.

The F-Test Results Table (Table R8.2) contains the following information:

Row 4: Average of each column of data.

Row 5: Variance of each column of data. Note, because the variance of the left data column was less than that of the right, you had to reverse the data for Variables 1 and 2.

Row 6: Number of data points in each column of data.

Row 7: Degrees of freedom (Consult one of the statistics books in the lab or LRC for an explanation.).

Row 8: The F statistic, which is the variable used to determine whether the difference between the two variances is significant.

Row 9: The one-tailed probability that these results could have occurred by chance.

Row 10: The value, which if exceeded by the calculated F (row 8) indicates that the difference between the variances is significant.

13. To start your t-test, select **Data** tab, then choose the **Data Analysis** icon.

14. Select your data as you did in steps 5-9 above except set your alpha to 0.05.

15. Look at your sample results (in Table R8.3), if your hypothesis is that one mean is different than the other, look at the two-tailed probability (row 13). If the value is less than 0.05 then you can determine that the means are significantly different

OR

If your hypothesis is that one mean is bigger than the other, look at the one-tailed probability (row 11). If the value is less than 0.05 then you can determine that the means are significantly different.

16. From the list of tests, choose either **T-test: two sample assuming equal variance** or **T-test: two sample assuming unequal variances**, depending on the **F Value** compared to the **critical value**. Click **OK**.

17. If your means are significantly different, report the result as "The mean speed of mice fed Diet A was significantly higher than that of mice fed Diet B (t-test, t=2.191, p = 0.026)" i.e., making sure to indicate the value of t and the probability of getting these values.

The t-test Results Table (Table R8.3) contains the following information:

Row 4: Calculated means for each group.

Row 5: Measure of variability or spread for each group.

Row 6: Number of subjects in each group.

Row 7: Combined variance because F-test was not significant.

Row 8: Means are tested against hypothesis of equality (i.e., the average speed of mice fed Diet A = the average speed of mice fed Diet B).

Row 9: Degrees of Freedom—Refer to a statistics book in the lab or LRC.

Row 10: The test statistic that the test generates—is its absolute value (row 10) larger than t- Critical (row 12 or row 14 depending on your hypothesis)?

			Row
t-Test: Two-Sample Assuming Equal Variances			1
			2
	Diet A	*Diet B*	3
Mean	2.916667	4.333333	4
Variance	0.641667	1.866667	5
Observations	6	6	6
Pooled Variance	1.254167		7
Hypothesized Mean Difference	0		8
df	10		9
t Stat	-2.19104		10
P(T ≤ t) one-tail	0.026621		11
t Critical one-tail	1.812462		12
P(T ≤ t) two-tail	0.053242		13
t Critical two-tail	2.228139		14

Table R8.3 | Sample Results From t-Test

Row 11: This is the probability that the means were this different when in reality they were the same. If this probability is very small ($p < .05$), then we tentatively (as always) conclude that they are different. . For example, if one group is given a high protein diet and the other is not, you really are interested only if the mean speed of mice in the experimental group is significantly greater than that of the control group.

Row 12: Critical t-value for Row 11.

Row 13: . For example, if you are testing mean time till effect for two anesthetics, then a significant difference is what is important, not that specifically A works faster than B (unless of course you are the manufacturer of A or B!)

III. Paired t-Test

A. Purpose

This test is very similar in use and interpretation to the t-tests described above. However, it is designed for use when testing subjects that are paired. This occurs, for example, if one is using the same individuals for the control and the experimental condition (e.g. unexposed vs. exposed to radiation or fed Diet A then Diet B).

B. Requirements

As for the t-test, except subjects are pairs matched in some way.

C. Procedure

1. Select **Tools > Data Analysis > t-test paired two sample for means**.
2. Follow the same steps as those for the t-test, but… .
3. Choose the option **T-test: Paired two samples for means**.
4. Examine the results (Table R8.4) and report the results as you would with a t-test.

   All rows are interpreted the same way as for the t-test (see II within this section). The new term, the Pearson Correlation, refers to how much one variable varies with the other. It provides a measure (0-1) related to the question "Are those with higher scores in the first test (or the control) the same as those that have the higher scores on the second?" Values close to zero indicate little correlation (i.e., those with higher scores in the first test don't have the high scores on the second), values close to one indicate high correlation (i.e., those with higher scores in the first test also have high scores on the second test).

t-Test: Paired Two Sample for Means		
	Diet A	*Diet B*
Mean	2.916666667	4.333333333
Variance	0.641666667	1.866666667
Observations	6	6
Pearson Correlation	0.213200716	
Hypothesized Mean Difference	0	
df	5	
t Stat	-2.42857143	
P(T ≤ t) one-tail	0.029742657	
t Critical one-tail	2.015049176	
P(T ≤ t) two-tail	0.059485314	
t Critical two-tail	2.570577635	

Table R8.4 | Sample Results From Paired t-Test

IV. ANOVA

A. Purpose

This procedure tests whether the means (averages) of three or more groups are equal. When used on only two groups, its use and results are the same as for the t-test, except that the statistic generated (F) and the values to compare it to are different. The test works by looking at variability in the scores in the groups. It compares the variance of the scores among the members of a group (within group variance) to the variance among the groups (between group variance). If the variability caused by the factor (experimental variable) is greater than the variability within each group, then the factor had a significant effect, and the means are different.

B. Requirements

1. The dependent variable must be continuous, i.e. measured not counted.
2. The same individual is not assigned to both groups.
3. The dependent variable is normally distributed (again we will mostly ignore this).
4. The data from the groups are of equal variance.

C. Procedure

1. Open Excel.
2. Enter your data as in Table R8.5.
3. Select **Data Tab > Data Analysis icon > ANOVA:Single Factor**.
4. Enter the range for the entire set of data in the **Input Range** space (Figure R8.2).
5. Select **Grouped By: Columns** by clicking the circle.
6. Check the **Labels** box.
7. Set the **Output Range** to a cell to the right of your data. Output will look similar to that in Table R8.6.
8. Click **OK**.

The ANOVA Output Table (Table R8.6) contains the following information:

Row 1: Title of Test

Rows 5-7: Provides the number of subjects, sum, average and variance for each group.

Diet 0	Diet A	Diet B
2	2	5
2	3	4
2.5	3.5	6
3	4	5
1	2	4
2	3	2

Table R8.5 | Sample Data for ANOVA

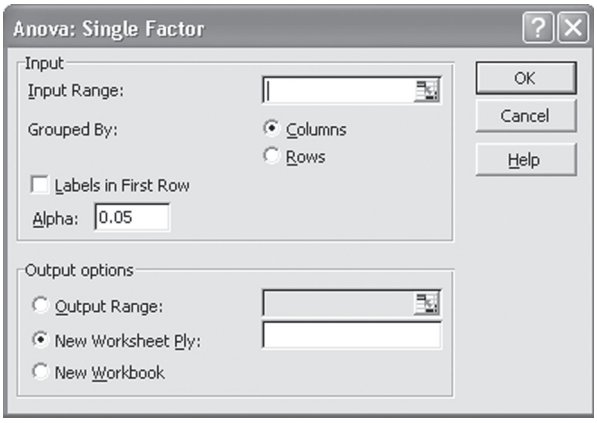


Figure R8.2 | ANOVA Window

	1	2	3	4	5	6	7
1	Anova: Single Factor						
2							
3	SUMMARY						
4	*Groups*	*Count*	*Sum*	*Average*	*Variance*		
5	Diet 0	6	12.5	2.083333	0.441667		
6	Diet A	6	17.5	2.916667	0.641667		
7	Diet B	6	26	4.333333	1.866667		
8							
9							
10	ANOVA						
11	*Source of Variation*	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
12	Between Groups	15.52778	2	7.763889	7.89548	0.004545	3.682317
13	Within Groups	14.75	15	0.983333			
14							
15	Total	30.27778	17				

Table R8.6 | Sample Results From ANOVA

Rows 11-15: Provides the parameters and statistics upon which the ANOVA was based. By looking at the p-value in column 6, we can determine whether the experimental variable had an effect. If $p < 0.05$, then the calculated F exceeds the F-critical and the probability that this was a chance occurrence is almost nil and the means are significantly different.

V. Correlation

A. Purpose

This technique allows you to determine if there is a relationship between one variable or set of scores and another. If there is a relationship, then as one increases or decreases, so does the other. In the case of this program, one can look for all possible correlations among a set of data involving multiple variables. A correlation of 0 means no relationship; a correlation of 1 is a perfect relationship.

B. Requirements

1. The measurement for all variables must be continuous (e.g., not counts) or nearly so.

2. There is no specific predictor variable that is being manipulated.

C. Procedure

1. Open Excel.

2. Enter the data as in Table R8.7.

3. Select **Data Tab > Data Analysis icon > Correlation**.

4. Enter the range for the entire set of data in the **Input Range** space.

5. Select **Grouped By: Columns** by clicking the circle.

6. Check the **Labels** box.

7. Set the **Output Range** to a cell to the right of your data. Output will look similar to that in Table R8.8.

8. Click **OK**.

At the intersection of each row and column is the correlation between the row and column variables. The correlation of a variable with itself is 1.

Test 1	Test 2	Test 3
80	82	78
72	71	70
65	55	48
95	89	78
95	98	97
77	74	75

Table R8.7 | Sample Data for Correlation

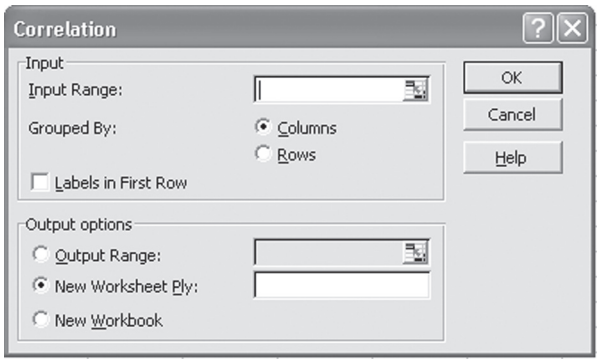


Figure R8.3 | Correlation Window

	Test 1	Test 2	Test 3
Test 1	1		
Test 2	0.955631	1	
Test 3	0.851168	0.958974	1

Table R8.8 | Sample Results From Correlation

VI. Regression

A. Purpose

A technique similar to correlation is regression. It, too, tests for a relationship between variables, but in this case one variable (or more) is used to predict the value of another. The advantage to regression is that it gives you more diagnostic tools and an equation that allows you to actually predict values. Typically the predictor variable is manipulated by the experimenter (e.g., change the levels of pH and watch the rate of change for a reaction). If this seems to resemble a trend line (see Reference , II.H) or fitting a line to data (see Reference , II.C), that's good—it is the same process. However, this procedure actually tests to see if the line is a good fit.

B. Requirements

1. The Y variable is normally distributed and continuous.
2. The X variable(s) are predictor variables that can be discrete.
3. The data are such that one could fit a line to them.

C. Procedure

1. Open Excel.
2. Enter data in columns with headers as in Table R8.9.
3. Select **Data Tab > Data Analysis Icon > Regression**, then click **OK**.
4. Enter the range for the variable you want to predict (the measured or dependent variable) for the **Y Range** by clicking on the little red arrow on that row of the dialog box, then selecting the cells, including the heading, for one column of data (Figure R8.4). Then press **Enter**.
5. Enter the range with the predictor variable for the **X Range** by clicking on the little red arrow on that row of the dialog box, then selecting the cells, including the heading, for one column of data. Then press **Enter**.
6. Check **Labels** (will be set to ignore the headings on your columns and use them for headings in the regression output).
7. Set your output range by clicking in the circle next to **Output Range** then clicking on the little red arrow on that row of the dialog box, then selecting a cell to the right of your data with plenty of empty space to the right and down. Then press **Enter**. The output will look similar to that in Table R8.10.
8. If you haven't already graphed your data and fit a line to it, shame on you! Here is a second chance, check the **Line Fit** plots (see Reference , II).
9. Ignore the other options for now. If you're curious, check with your instructor or the statistics texts in the lab or LRC.

pH	Rate of Uptake
7	90
6	83
5	72
4	61
3	54
2	43
1	30

Table R8.9 | Sample Data for Regression Analysis

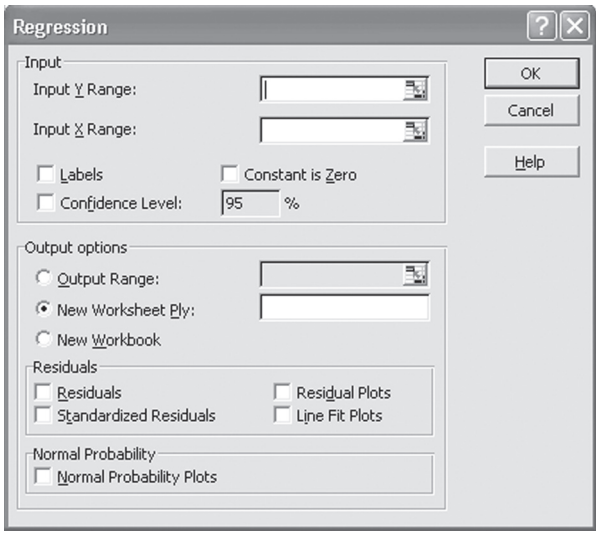


Figure R8.4 | Regression Window

10. Click **OK**.

The Regression Output Table (Table R8.10) contains the following information:

Rows 4-7: For our purposes, we will just look at the Adjusted R^2 and use it to interpret results as follows—if 0, the line does not fit the data at all; if 1, it is a perfect fit.

Row 8: Number of subjects.

Rows 11-14: Here is the information dealing with the calculation of the test as to whether there is a line that fits these data with a slope different from 0. If the significance of the F-statistic is less than .05 (last column, row 12), then the fit is unlikely to have occurred by chance and you can report a significant linear relationship between X and Y ($R^2 = 0.99$, $p < 0.05$ significance value).

1	SUMMARY OUTPUT								
2									
3	*Regression Statistics*								
4	Multiple R	0.997345117							
5	R Square	0.994697282							
6	Adjusted R Square	0.993636738							
7	Standard Error	1.715475777							
8	Observations	7							
9									
10	ANOVA								
11		*df*	*SS*	*MS*	*F*	*Significance F*			
12	Regression	1	2760.143	2760.143	937.9126	6.97E-07			
13	Residual	5	14.71429	2.942857					
14	Total	6	2774.857						
15									
16		*Coefficients*	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
17	Intercept	22.14285714	1.449842	15.2726	2.18E-05	18.41593	25.86979	18.41593	25.86979
18	pH	9.928571429	0.324194	30.62536	6.97E-07	9.095204	10.76194	9.095204	10.76194

Table R8.10 | Sample Results From Regression

Rows 17-18: These provide additional statistics to indicate how good the line fits and whether the intercept or slope (row 18) of the line are zero. Again, you should consult a statistics book or instructor if you wish to know more, but we can ignore this for now.

VII. Chi-square (χ^2)

A. Purpose

Chi-square (χ^2) tests whether a set of observed counts (frequencies) matches a set of expected counts or frequencies. In the version of the test illustrated in this example, what is being tested is whether the different levels of the factor in the columns and the factors in the rows influence each other.

B. Requirements

1. The data are counted not measured.
2. Each subject or item must fall into one category (box).

C. Procedure

The test works in three stages:

1. Determine the expected values.
2. Determine the test statistic, the chi-square, χ^2.
3. Compare the test statistic to a table that determines the probability of achieving that number by chance.

These steps are simplified when using .

To determine the expected values:

1. First determine the row and column totals. In the example below, that was achieved by using the sum function in the cells marked 1 and 2 (the gray-shaded area) and copying those functions to the other cells at the ends of the rows and columns.
2. Each expected value is equal to the Row Total * Column Total / Grand Total. The logic is simple. The expected value is set to the proportion of the total that would be expected in each cell if the ratios in the cells in each column were the same. In our example (Table R8.11), this is determined by placing formula 3 in the upper left hand cell of the block that holds the expected values and copying that formula to the other cells. Let's look at that formula. The "=" simply starts the formula. D5, B8, D8 are the addresses of the cells we need to calculate the expected value. But to be able to copy the formula to other cells we need to fix certain parts of the addresses. The same cell is always used for the grand total so a "$" precedes the column letter and row number of that cell making it an absolute address. The column is fixed for the row totals (but the row is allowed to vary) and the row is set for the column totals (but the column is allowed to vary). This is accomplished by placing a "$" before that part of the address. It is easiest to then enter this formula into the upper left cell of the expected block and then copy it to the others. For more information in this area, refer to Reference , II.

	A	B	C	D	E	F	G	H
1	Example of a Chi-Squared test							
2								
3	**Actual frequencies observed**							
4								
5		**Juveniles**	**Adults**	**Row Totals**				
6	Snakes	58	35	93				
7	Cats	11	25	36				
8	Dogs	10	23	33				
9	**Column Totals**	79	83	162				
10								
11								
12	**Expected frequencies**							
13		**Juveniles**	**Adults**	**Row Totals**				
14	Snakes	45.351852	47.6481	93				
15	Cats	17.555556	18.4444	36				
16	Dogs	16.092593	16.9074	129				
17	**Column Totals**	79	83	162				
18								
19								
20	**Chi-Square Value:**	16.164035						
21	**Probability:**	0.0003089						
22	**df:**	2						
23								
24								

The formulas in these cells are simple sums **=SUM(B6:C6)** either across or down the columns

The formulas in these cells calculate the expected values for each cell using the formula **(Row Total)*(Column Total)/(Grand Total)** as follows:

=+$D6*B$9/$D$9; **=+$D6*C$9/$D$9**
=+$D7*B$9/$D$9; **=+$D7*C$9/$D$9**
=+$D8*B$9/$D$9; **=+$D8*C$9/$D$9**

Notice the use of absolute addresses **($)** so you can simply enter the first formula in Cell B14 then copy it to the other cells.

The formulas in these cells are simple sums **=SUM(B14:C14)** either across or down the columns

The formula in this cell **=CHIINV(B21,B22)** calculates the values from the d.f and probability

The formula in this cell **=CHITEST(B6:C8,B14:C16)** calculates the probability that the actual results occurred by chance. A value below 0.05 means that the differences among groups are most likely real.

The formula in this cell **=(COUNT(D6:D8)-1)*(COUNT(B9:C9)-1)** calculates the number of degrees of freedom **(rows-1*cols-1)**. It represents the number of categories that you could change given that you know the sums of the rows or columns. This is needed for the test.

Table R8.11 | Sample of Chi-Square Test

Reference

Literature Research Survival Guide

Written by Tarren Shaw and Anne Prestamo

Scientists regularly use research articles as a way to justify methods and support ideas in their reports. For what can you use research articles? You can add support and information to your discussion section and earn extra credit! Referring to research articles in your lab report demonstrates that you've taken the time to think about your conclusions and compare them to existing scientific studies. But where should your search begin?

For every investigation there is a list of terms located in section B that may be useful in writing your lab report. Many of these terms make good places to start a search for research articles and web pages. Of course there may be other, more general terms you'd like to use. Ask yourself, "What topics are we investigating? What is our lab about?" For example, Investigation 14 explores how guppies attract mates. A search using "Guppies" and "Sexual Selection" will yield many useful results.

I. Searching for research articles

A. Beginning your search

Open Internet Explorer and navigate to the library homepage: www.library.okstate.edu.
Select the link for "**Indexes and Databases**" located in the **Find** menu (see Figure R9.1). You will see a list of the hundreds of databases that are available for you to search. This can seem overwhelming so we will focus our search using the **EBSCO Full-text Database**. This database allows you to narrow or broaden your search by combining or excluding search terms in additional rows.

- **AND** will narrow your search: "Guppies AND Sexual Selection" will find the articles that contain *both* of these terms.
- **OR** will broaden your search: "Guppies OR Fish AND Sexual Selection" will find articles about sexual selection regarding either guppies or fish.
- **NOT** will narrow your search: "Guppies NOT Pet Stores" will exclude any articles about guppies that refer to pet stores in the article.

Additionally, EBSCO allows you to select which part of the articles you would like to search by changing the search fields. You can try searching in some of the suggested fields listed below.

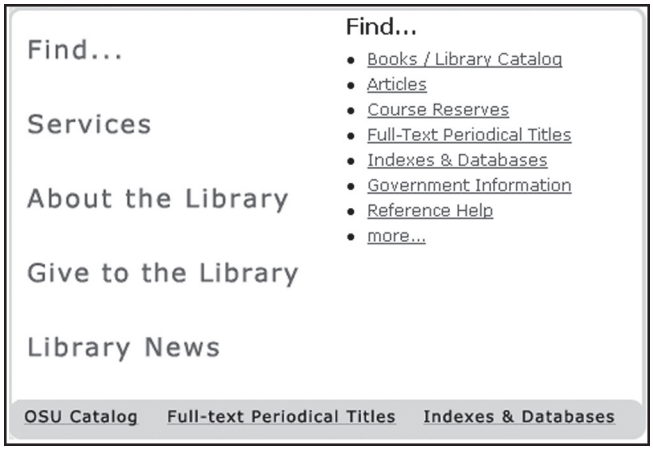


Figure R9.1 | Library Find Menu

- **Default Fields:** This field searches the combined fields of abstract, title, author, and subject for your term. Default Fields provides a wide variety of results and can be a good starting point for many searches.
- **Abstract:** The abstract is a summary of the article. Searching within this field increases the probability that the term will be discussed in the research of the article.
- **Subject Terms:** When an article is published, the author selects a few keywords that aid in identifying the main subject of the article. This field searches these keywords to find articles that deal with the subjects you are searching.
- **All Text:** It is the broadest search field and searches every part of the article including the abstract, and citation section for your selected term.

B. Using EBSCO

Start by selecting the letter E from the A-Z list at the top of the library web page. This will take you to all the databases that start with the letter E. From this list, select **EBSCO Full-text Databases** (see Figure R9.2).

The first screen in EBSCO asks you to select the databases from which you would like to search, but the default is set so that it searches the Academic Elite database. This is what you want. Click on the **Continue** button.

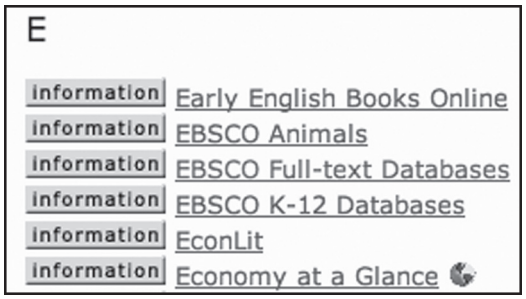


Figure R9.2 | Selecting EBSCO

C. Searching with EBSCO

Let's search for articles using some terms from Section B of Investigation 1. To find the most results, start by searching **Default Fields**. Figure R9.3 shows an example of a search using the term **crypsis**. You may want to narrow your search to a specific part of the article such as the title or the abstract. You can do this by selecting a different field to search from the drop-down field menu (see Figure R9.4). Once you have your terms and fields selected, click on the **Search** button to start your search.

A search for the term **crypsis** in the **Abstract** field yields 11 results (see Figure R9.5). Double clicking on the title of the article will link you to the web page that contains the abstract, text, and reference information for the article.

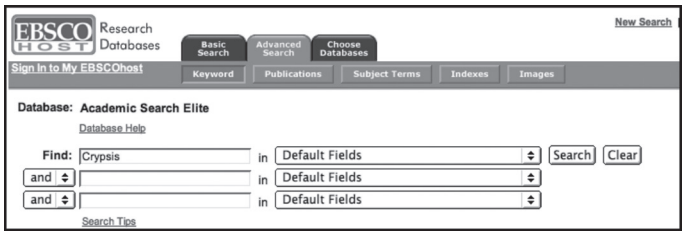


Figure R9.3 | Searching EBSCO

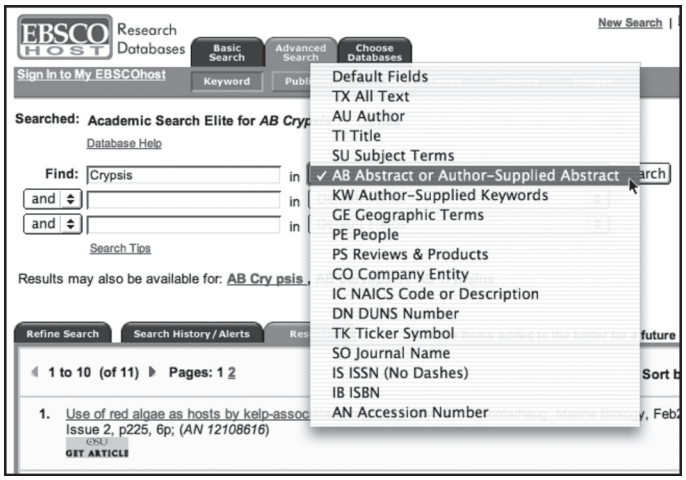


Figure R9.4 | EBSCO Search Field Menu

Once you select an article by double clicking on its title, EBSCO allows you to search for similar articles using the keywords listed under the **Subject Terms** heading (see Figure R9.6). Take a look at the terms used, they can help you learn what terms are useful for searching and finding more articles. Clicking on the terms will provide you with a list of articles that share this subject term. Of course you can search for any combination of terms you'd like, in any field you'd like. Sometimes your search may come up with hundreds of articles. To narrow down your choices, you will want to add another term to search with. With a little practice you'll get a better idea of which fields produce the most useful results.

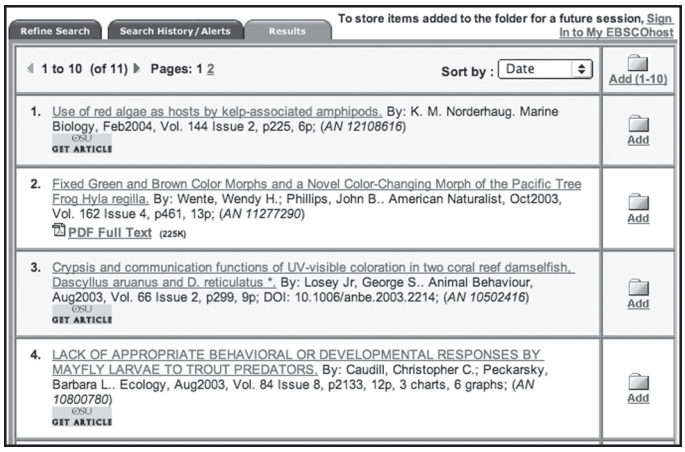


Figure R9.5 | Results From an EBSCO Search

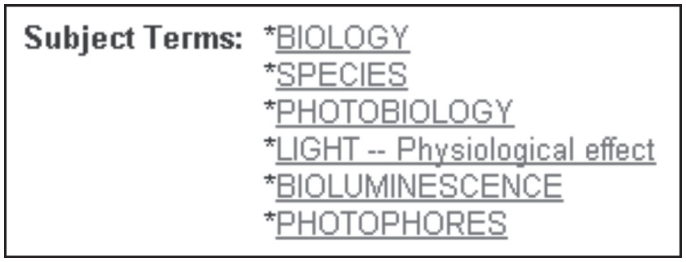


Figure R9.6 | Example Subject Terms

Reference

II. Using the World Wide Web for research

Information is at our fingertips! Thanks to the World Wide Web, we now have access to huge amounts of information within seconds. This easy access to information can be a great tool when conducting research, but it's important to keep in mind that the Web is not necessarily the magic answer to all of your research needs.

Before turning to the Web to do research, be sure that you have a clear understanding of your topic. You need to know some basic information about your topic in order to effectively use the Web, and to evaluate the information you find.

A. Evaluating web page information

There are some web sites that the Library subscribes to that are tools for doing research. There are web sites that are created by faculty at universities, sites that are created by businesses and companies, and sites that are created by organizations and individuals.

Just because information is found on a web page does not mean it's true or correct!

When you use the web for research you need to think critically and evaluate the information you find. This is really no different than what you do with printed material. Think about these examples. When you were in 4th or 5th grade the *World Book Encyclopedia* was probably fine to use as the source for a report. By the time you got to high school, you needed sources with more complex and detailed information. If you need an article from a newspaper, which would you believe to be more reliable—the *Wall Street Journal* or the *National Enquirer*?

These examples are so obvious that they may seem ridiculous, but evaluating web pages is really no harder, if you know what to look for.

There are five primary criteria to consider when evaluating a web page. They are:

- **Authority—**Who is the author? What qualifies that person to write on this topic?
- **Accuracy—**Is the material clearly and logically presented? Are sources of the information cited? Are there spelling or grammatical errors?
- **Currency—**Does the page include the date it was created and/or the date it was last updated? If the page contains statistical information, is it clear when the data were collected? Do links to other web pages work?
- **Scope and Depth—**Does the page include a statement of purpose and the topic(s) addressed? Does the page succeed in addressing these topics or has something significant been left out? Are there "Under Construction" graphics or messages? These usually indicate that the information provided is incomplete.
- **Objectivity—**Who is presenting this information? If it's an organization, what is their purpose? Is this page trying to advance a particular cause or is it trying to sell something?

The way in which you ask these questions will depend on the type of information you need. For example, if you want purely factual information, then you need to find web pages that are free of bias. In some cases, however, you may want to find out what the opposing viewpoints are on a given issue. In that case, web pages that represent differing opinions on the issue may be very informative.

B. Searching the Web

Finding what you need on the Web can be a real challenge. New web sites and web pages appear constantly, and there's no single place that organizes all of the content.

When you're looking for something specific, finding exactly what you need can be very time consuming. There's so much out there that it's hard to know where to start. One way is with a search engine.

Search engines are computerized indexes to the World Wide Web. They allow you to use keywords to search for pages that contain information on your topic. There are many to choose from, they all work a little bit differently, and some index more of the Web than others. Try using several until you decide which provides the best results for you.

The most widely used search engines are:

Google: *http://www.google.com*

MSN: *http://www.msnsearch.com*

Yahoo: *http://www.yahoo.com*

III. Finding books in the library

In addition to using information from research journals and web pages in your lab report, you may also want to utilize technical books that deal with your topic. The library has over one million volumes but using this guide can help you easily find what you're looking for.

Open *Internet Explorer* and navigate to the **OSU Library Catalog** page: *http://osucatalog.library.okstate.edu/*. There are several search types to choose from, but the **Keyword** and **Basic** searches will probably be the most useful.

A. Searching by keyword

If you don't know the title or author of a book you're looking for, this is a good place to start. Instructions and tips for searching are listed just below the search window. Similar to searching with EBSCO, you decide for what term(s) and field(s) to search. While the method is a little different, the library catalog can be searched using AND, AND NOT, and OR to join several terms. Additionally, a "?" placed at the end of a word will find different forms of that word. For example, the keyword "geneti?" will retrieve anything with the keyword "genetic," "genetics," and genetically."

B. Searching with Basic

If you know the Author or Title of a book or journal you'd like to find, Basic is the best way to find it. A Basic search is easily performed by following the instructions and tips listed below the search window.

Both Keyword and Basic searches will provide a list of titles of the books selected. Clicking on the title will provide more specific information such as the call number, whether the book has been checked out or not, and additional subject terms to search within. When you know the call number of the book, use the chart below to find its location in the library.

Call Numbers	Location of Books/Bound Journals
000-329	3rd Floor
330-499	4th Floor
500-624	Basement
625-699	1st Floor
700-999	4th Floor

IV. Creating a literature cited section

When you prepare your lab reports, every source you use must be listed in your Bibliography or Reference List. This is true for web pages, just as it is for books, magazines, and journals. For BIOL 1114 you need to follow the format outlined in Section GVII.

Reference