<u>**This article reprinted from:**</u>

Ratterman, D.M., J. Watrous, and D. Lurie. 2006. Integration of biology and statistics education (IBASE): measurements of cells and organelles in biology lab to produce large data sets that can be analyzed in statistics classes. Pages 217-237, *in* Tested Studies for Laboratory Teaching, Volume 27 (M.A. O'Donnell, Editor). Proceedings of the 27th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 383 pages.

Although the laboratory exercises in this proceedings volume have been tested and due consideration has been given to safety, individuals performing these exercises must assume all responsibilities for risk. The Association for Biology Laboratory Education (ABLE) disclaims any liability with regards to safety in connection with the use of the exercises in this volume.

The focus of ABLE is to improve the undergraduate biology laboratory experience by promoting the development and dissemination of interesting, innovative, and reliable laboratory exercises.

Visit ABLE on the Web at:
http://www.ableweb.org

Integration of Biology and Statistics Education (IBASE): Measurements of cells and organelles in biology lab to produce large data sets that can be analyzed in statistics classes.

Denise Marie Ratterman[1], James Watrous[1], Deborah Lurie[2]

[1]Department of Biology
Saint Joseph's University
Philadelphia PA 19131-1395
FAX 610-660-1832
dratterm@sju.edu; 610-660-1822 (DMR),
 jwatrous@sju.edu; 610-660-1829 (JW)

[2]Department of Mathematics and Computer Science
Saint Joseph's University
Philadelphia PA 19131-1395
FAX 610-660-3082
lurie@sju.edu
610-660-3345

IBASE Website: www.sju.edu/cas/ibase

Abstract: This lab was designed to promote the integration of a freshman biology lab and an Applied Statistics course. Students collect data sets to be used in statistics for examples and projects, and then present their findings in biology lab. Students learn how to determine the actual size of cells using calipers for chicken eggs, microscopes with ocular micrometers for onion root cells, microscopes with digital cameras/measuring software for red and white blood cells, and rulers for measurements of organelles and bacterial cells on electron micrographs. Students are also introduced to SPSS software to be used in both classes.

Keywords: integration, statistics, micrograph, cell size, micrometer, microscopy, SPSS, units

Contents:

Introduction

This project represents cooperation between the biology and mathematics faculty to integrate the early training in data collection and analysis in the biology laboratory with an introduction to statistics and especially descriptive statistics. Students are enrolled in both their first biology course (Bio I: Cells) and an Applied Statistics course during the first semester of their freshman year. Students collect data sets that are then used in the statistics class as examples and for projects. Methodology learned in the statistics class is used by the students to examine the data and present their findings in biology lab, addressing some biological questions. The implementation of this IBASE (Integrated Biology and Statistics Education) curriculum has been funded by the National Science Foundation (DUE award # 0309751).

This workshop introduces the laboratory work that the students complete in the first two 3-hr lab meetings in Bio I: Cells. These data are then combined to form large data sets (from eight lab sections). Student teams analyze their data set for one of six project themes in their statistics class and then present their results using descriptive statistics to their biology lab section in a later lab period.

Students use a variety of measuring approaches to measure cells:
- Eggs are measured with calipers (length and width in mm) and scales (mass in g)
- Onion root cells are measured using ocular micrometers in student microscopes
- Red and white blood cells are measured using digital cameras/measuring software
- Bacteria, nuclei, and mitochondria are measured with rulers on electron micrographs

Basic lab concepts and skills are also introduced:
- Unit prefixes and conversion between units, especially mm to um
- Use of digital scale, calipers
- Basic use of compound microscope
- Measuring using ocular micrometer; Calibration of ocular micrometer
- Conversion to actual size on micrographs (using scale bar or magnification)
- Keeping a record of data (starting a lab notebook)
- Brief introduction to types of microscopy *(optional)*
- Brief introduction to domains/kingdoms of life *(optional)*

Student Outline

Overview

As you sit down at a lab bench today you are picking your teammates for the semester. Pick teams of THREE. If the class size is 19, there may be one team of four and five teams of three, but otherwise teams of three or two only. At least one member of each team should be in Applied Statistics this semester or had it last fall. We will be collecting some biological data in labs for use in this statistics class. Note which team number your team has – each team will have a specific project using the data we collect this week and next.

1st week outline
- 1st hour – Welcome, pick teams. Collect egg data set data and enter into SPSS.
- 2nd hour – Teacher introduces unit conversions, the use of microscope, Lab 1 data sets; students get comfortable using the microscope
- 3rd hour – Teams work on collecting data for our three main data sets for Lab 1

2nd week outline – teams continue Lab 1 more independently
- 1st hour – Computer Classroom visit – introduction to SPSS tutorial
- 2nd & 3rd hour – Teams work on collecting data for our three main data sets for Lab 1
- With any extra time – gain experience with the microscope looking at the live protists /other slides available, visit the types of microscopy display, and learn the domains/kingdoms of organisms.

Team projects on the data sets are listed on the next page. Students who are in Applied Statistics will work with the same data sets as they will for bio lab – so make sure you remember your team number to tell your statistics teacher. Your team will present in bio lab in November on your project.

Team Projects

All teams will collect data for all four data sets. Once the data are compiled for all lab sections, teams focus on one project.

Egg Data Set

Used as an example data set for Applied Statistics analysis. You collected these egg length, width, and mass data so can lend your experience to the analysis of it as you learn statistical analysis.

Micrograph Data Set

Actual size for whole cells of bacteria and for two eukaryotic organelles (nuclei, mitochondria)

Team 1 examines the sizes of a sample of prokaryotic cells. How big are bacteria? Does the typical size (central tendency) and variability appear different for the different groupings (grouped by shape: spheres, rods, spirals)? How does the size of bacteria (whole prokaryotic cells) compare to the size of mitochondria (one eukaryotic organelle)? Be certain to introduce what is thought to be the origin of mitochondria in your presentation. Which type of bacteria are the mitochondria most similar to in size and shape?

Team 2 examines the sizes of nuclei and mitochondria. This is from a sample of micrographs. Was there a great deal of variability in size? Did our measurements from the same micrograph vary? What is the average size of each organelle? How many of each could be laid end to end across the length of a typical animal cell? A typical plant cell? What are the sizes of other eukaryotic organelles? How do these compare to the size of a nucleus? If you find background information on the subject, also tell us how many of these organelles are found in certain cells.

Blood Data Set

Diameter of red blood cells (rbc) and white blood cells (wbc) in normal blood and blood from patients with a blood disorder. Also collected are some frequency data on number of wbc per field.

Team 3 examines the diameter of red blood cells. What is the typical size of a red blood cell? How does this compare to what is given in texts for the typical size of an animal cell? Was there a large amount of variability in diameter? How does the function of a red blood cell relate to its size? Did the profile of red blood cell diameter change in the iron-deficient anemia? Does shape change with sickle cell or pernicious anemia?

Team 5 examines the diameter of wbc. What is the typical size of a white blood cell? Did the different categories of white blood cells (neutrophil, eosinophil, etc) show similar diameters? Are these types of wbc different in both form and function? Did the frequency of white blood cells per field change in the mononucleosis and leukemia patients when compared with the healthy donors?

Root Data Set

Length and width pairs for individual cells in the region of new cell formation (meristem) at the tip of the growing onion root, compared to cells farther up the root tip and to mature cells of different types from top portion of the scallion (green onion) root.

Team 4 examines the difference in size and shape (if any) between the very young onion root cells in the meristem region (where new cells are formed for the root to grow) and older cells. Did the profile of widths change as cells were sampled farther up the onion root (from younger to older cells)? Did the lengths change? How do these compare to the widths and lengths found for mature ground tissue cells much farther up the scallion root? What type of processes do you think might have occurred as these cells got older?

Team 6 compares the different types of mature root cells from the scallion root. Are the xylem, endodermal, and ground tissue cells similar in diameter and shape? Are they distinguishable from one another? Is there more uniformity in any of these cell types? All were first formed as the new cells in the root tip. Are the mature cells as similar in size and shape as the newly-formed cells in the meristem (root tip)? Estimate how much larger each type (on average) is than an average meristem cell. Do you think there might be some control over the changes that occur for a particular cell as it matures?

Our first data set: EGGS

To begin class, we will collect size and mass measurements from chicken eggs. The data collected by all the Cells Lab sections will be compiled and made available to Applied Statistics classes for

analysis. What do eggs have to do with cells? Consider a newly-hatched chick. This is a multicellular animal, composed of many cells. Where did these cells come from?

> The Cell Theory
> 1. All organisms consist of one or more cells
> 2. The cell is the basic unit of structure for all organisms
> 3. **All cells arise only from pre-existing cells**

They must have come from previously-existing cells by cell division. But, even if we trace every cell of the chick back to one original cell (called the zygote), where did this cell come from? It was formed from the union of a cell from the father and a cell from the mother of the chick. This event was the fertilization of the egg by a sperm. This reminds us that the egg itself is a cell.

Although most cells are microscopic, we do not need a microscope to view a chicken egg. On your team bench are some samples of chicken eggs. They are all quite large but are not identical in diameter and overall size. Variation is the norm in biological systems. To begin to learn about variation and how it is examined, we will measure the eggs and collect a large data set.

Collecting Egg Measurements

1. Each team has 6 eggs so that each team member can measure 2 of the eggs. To measure the length and width of eggs, use a digital caliper. Before starting your measurements, make certain your team's caliper is set to mm rather than inches. A button on the side is used to set zero before opening the caliper to span the distance to be measured. Measure the length and width (diameter) of each egg, adjusting the caliper to record the largest distance the egg measures in each of these dimensions. These are fresh eggs so treat them with care.

2. To measure the mass (weight) use the portable electronic scale at your bench. Check that this is showing grams (g) not ounces (oz). Place a weigh boat or measuring cup on the scale and tare this (set to zero) before placing the egg in the weigh boat.

3. Record your team's data in your own observation notebook in a table similar to Table 1 below. Your instructor will lead your class in entering the data on SPSS to give you an introduction to this program.

Table 1. Example Egg Data

section	Team	Caliper number	Egg number	Size des. (M,L,XL)	Length (mm)	Width (mm)	Mass (g)
122	4	7	186	M	54.12	41.06	49.7
122	4	7	150	L	49.47	45.26	56.9

Introduction to Units & Unit Conversions

Your instructor will give you an introduction to units of measurement. You need to learn the meaning of the prefixes and how to convert from one unit to another (such as from μm to mm). The ones we use *all the time*:   milli m 10^{-3} micro μ 10^{-6} nano n 10^{-9} kilo k 10^{+3} Practice until this is easy and natural to you.

Introduction to the Light Microscope

Since most cells are microscopic, the microscope is an invaluable tool in the study of cells. Your instructor will review the use of the microscope with you. An illustration of a typical microscope and parts can be found at each table. You are required to know the major parts of the microscope and their function for the next lab practical. These slides are in your team box for practice focusing:

- Letters on tiny slips of paper under the coverslip (easiest to start with)
- Human or Mammalian kidney section
- Privet (*Ligustrum*) leaf section
- *Paramecium* (wm = whole mount) or other protist such as *Amoeba* or *Chaos*

How do I focus in without breaking the slide?

1. First, use the lowest power and place the slide with the sample in the center of the stage. Look into the scope and see if you can see the color of the sample – moving the slide back and forth can help you find the blurry color moving across the field. Try to position it in the center of the field.

2. With the 4x or 10x objective, use the <u>coarse</u> focus to focus in the sample. Get this clearly focused in and place the structure/cell in the center of the field using the stage controls. You can be bold when using the coarse focus for the 4x and 10x objectives – these will not be able to bash into the slide (they stop short). If necessary, go all the way down and start back up to find the focus.

3. Once clearly focused with the 10x objective, swing in the 40x objective. Focus at 10x, then swing in – do NOT stop and move the stage down before swinging in the higher power objective. Only go from one objective to the next highest, do not skip an objective. Then, focus <u>only</u> with the fine focus. Never use the coarse focus with the 40x, 60x or 100x objectives. One good strategy is to hold the fine focus knob and turn it toward you if things are getting more blurry, then turn it back to the starting point and turn to the other direction. It should take less than a full turn either direction to find clear focus. If it is taking more than one turn of the fine focus, go back to focus in very clearly with the 10x objective and then return to the 40x. If you lose the sample or have trouble focusing, go back to the 10x objective and re-focus in. Then, go back to the higher power.

Micrograph Data Set

So, chicken eggs are cells. What about bacteria?

The Cell Theory

1. **All organisms consist of one or more cells**
2. The cell is the basic unit of structure for all organisms
3. All cells arise only from pre-existing cells

Each bacterium is a single cell, such that the single cell *is the organism*. How do prokaryotic cells differ from eukaryotic cells? One difference usually mentioned is that prokaryotic cells are typically smaller. To see how much smaller, we will compare the size of some bacteria to the size of single organelles of eukaryotic cells, the mitochondrion and the nucleus. This will also give us a chance to become familiar with the size of these two large organelles. Do bacteria have nuclei and mitochondria inside their cells? Do animal cells? Plant cells?

Collecting Micrograph Measurements
1. Each team table should have six micrographs, identified by a label:
      Bacteria - Magnification given   Bacteria - Scale Bar
      Mitochondria - Magnification   Mitochondria - Scale Bar
      Nucleus - Magnification given   Nucleus - Scale Bar

2**.** Each team member should chose one micrograph with magnification and one with a scale bar to measure. You will need to learn how to convert from the mm measurement read from the ruler on the micrograph to actual size in both these situations.

3. Measure the organelle or cell using a ruler. Record your measurement in mm, not cm.
      a. For mitochondria or nuclei – measure the length (longest dimension) of the organelle. There may be several mitochondria in a single micrograph, choose any you can clearly see the full length of.
      b. For bacteria, use a ruler to measure the length (longest dimension) for a single cell. Some micrographs show many bacteria; pick any one you can see the full length of clearly. Also note what shape the bacterium has. Most bacteria can be described as falling in one of these three categories:
            i. Sphere - called coccus (*kok' ûs*, cocci, *kok' sî ,* pl) – round or oval-shaped
            ii. Rod –   called bacillus (*bâ sil' ûs*, bacilli, *bâ sil' î* , pl) – rod-shaped (elongated)
            iii. Spiral – curved or spiral (corkscrew). Includes curved rods (vibrio, vibrios pl), rigid spirals (spirillus, spirilla pl) and flexible spirals (spirochete, spirochetes, pl).

4. Also collect this needed information from your micrograph:
      a. For micrographs with magnification, read the legend to find the magnification used and note this (such as 6000x or 10,000x).
      b. For micrographs with a scale bar, measure the scale bar to the nearest 0.1 mm (read the mm and then estimate to nearest tenth of the unit) and note this measurement and what is given for the actual size of the scale bar.

5. Convert your measurement to actual size in μm
      a. Converting to actual size when magnification is given. To convert to actual size, divide by the magnification. Watch those units! The magnification itself has NO UNITS because it is a ratio. Then, convert your answer from mm to μm. Or, you may want to include a conversion factor in your equation to convert the units right away. Do you divide or multiply by 1000?
$$\frac{\text{(size in mm measured on micrograph)}}{\text{(magnification)}} = \text{actual size in mm}$$
      b. Converting to actual size using a scale bar. The actual size that corresponds to the drawn scale bar is given. Divide your cell or organelle's length by the scale bar length to determine "how many scale bar lengths" it is equal to. Since you measured both in mm, these units will cancel. Now multiply this by the actual size of the scale bar. Your answer will be the organelle/cell's length in the same units as shown for the scale bar.
$$\frac{\text{(size in mm measured on micrograph)}}{\text{(measured size of scale bar in mm)}} \times \text{(actual size of scale bar)} = \text{actual size}$$

A note about significant digits: Often, the bar is drawn to show the distance for an easy unit of measure such as 1 μm. It is common to show this as 1 μm rather than 1.0 or 1.00 even if it does have that level of precision. Perhaps this is to save space. Regardless, do not drop your digits – keep the same number of digits in your measurement with the ruler because the scale bar is more precisely measured than shown in its digits.

6. Organize your team's data in your own observation notebook in a table similar to the example below. Have your team enter the data directly into the SPSS spreadsheet when the computer for this is available. Be sure to save after your entry. Proofread your values. Are they similar to the other groups? A large difference may indicate a conversion error you'll need to track down.

Table 2. Example micrograph data

section	Team	Specimen*	Micrograph#	Length (mm)	Actual Size (μm)	Shape**
122	4	1	89	26.0	2.2	3
122	4	1	80	6.0	1.5	1

*specimen has value labels defined as 1= bacterium 2=mitochondrion 3=nuclei
**Shape: 1= sphere (round) 2= rod 3=spiral (or curved) this is recorded for bacteria
    For mitochondria and nuclei, enter 99 in the Shape column (99 for "not recorded")

Blood Data Set
Because many students are interested in medicine, we cannot forget to examine human cells!

The Cell Theory
1. All organisms consist of one or more cells
2. **The cell is the basic unit of structure for all organisms**
3. All cells arise only from pre-existing cells

   There is a sample of human blood on one of the slides in your team's box. There are also samples of blood from individuals with a few disorders such as anemia, leukemia or mono.

Collecting Blood Cell Measurements

1. Take your team's slide box over to a digital camera/microscope station when it is available. Have your team together at that station for these measurements. Instructions at the station will guide you in using the software for visualizing the cells on the computer. Using the commands shown on the instructions, continue with step 2.

2. Find the stage micrometer slide in your team's slide box. The stage micrometer is an actual 2 mm ruler that is under the coverslip. At the lowest power, the whole 2 mm is in view (Fig. 1). When viewed at high power (40x or 60x objective), you may only see the first few 0.1 mm divisions. The first 0.1 mm is divided into 0.01 mm units. Since 0.1 mm is equal to 100 μm, each division is then actually 10 μm in this earliest region.

3. Using the stage micrometer slide, focus on the image of the small ruler (Fig. 1). Use the objective with lowest power first. Once clearly seen, swing in the next higher power objective. Use only the

fine focus at this point. Then, the next. Have your image focused with the 60x objective in place before capturing images. Capture an image.

Figure 1. The stage micrometer as seen in the field of view. It is actually 2 mm in length, marked in 0.1 mm units. The first 0.1 mm is divided into 0.01 mm units. It will move when you move the slide using the mechanical stage controls.
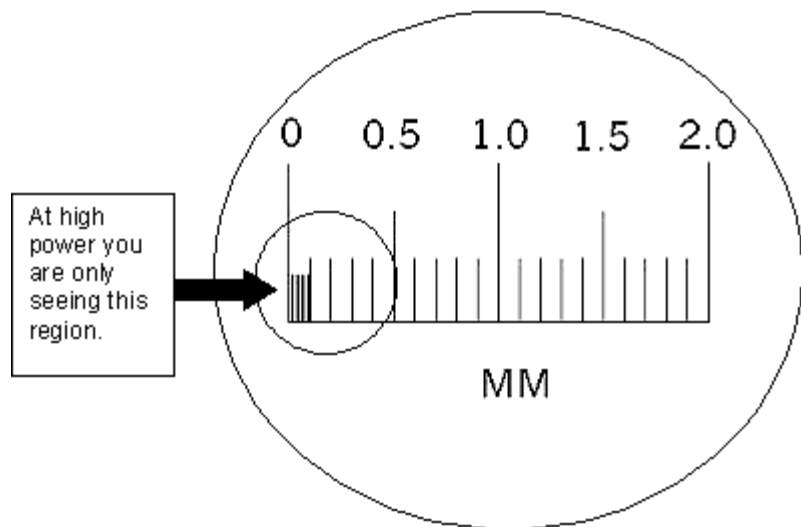
4. Have your team identify a distance on the image that is actually 100 μm. Enter the calibration window using the commands shown at your station and then drag out this distance. This step tells the computer how many pixels on the image is equal to this actual distance.

5. Next, focus on the human blood smear specimen. This is our healthy blood sample. Have your image focused with the 60x objective in place before capturing images. You will be looking at the two major categories of blood cells: erythrocytes (red blood cells) and leukocytes (white blood cells). The white blood cells do not appear as round and look distinctly different from the red blood cells. The white blood cells have a darkly stained (usually purple) nucleus. You will see many more red blood cells than white blood cells, but if you scan around you will see white blood cells.

6. Capture a few images until you have found at least three red blood cells and three white blood cells that can be measured cleanly. There is a display at the station showing some of the problems to avoid when choosing blood cells to measure.

7. Enter the measurement window using the commands shown at your station. Drag out the diameter of cells to measure them – the computer will convert the pixels dragged out into actual size in micrometers. Have your team measure diameters of three red blood cells and three white blood cells.

8. For each of the white blood cells you measured, use the dichotomous key and pictures at your station to determine the type of white blood cell each is.

9. Next, focus on the iron-deficiency anemia blood sample. Have your image focused with the 60x objective in place before capturing images. Collect an image.

10. Measure the diameter of three red blood cells from the iron-deficiency anemia sample. We will not be examining the white blood cells of this sample.

11.   Organize your team's data in your own observation notebook in a table similar to the example below. Have your team enter the data directly into the SPSS spreadsheet when the computer for this is available. Be sure to save after your entry. Proofread your values.

Table 3. Example blood diameter data

section	Team	Cell type*	Diameter (μm)	wbc_type**
122	4	2	11.76	2
122	4	1	7.27	99

*cell type: 1= healthy red blood cell   2=white blood cell   3= anemic red blood cell
**wbc_type: 1=eosinophil 2= neutrophil 3= basophil 4= lymphocyte 5=monocyte
   For red blood cells, enter 99 in the "wbc_type" column.

12.   At your team microscopes, look at the sickle cell anemia and pernicious anemia blood smears. What is different about these red blood cells compared to the blood from the healthy subject? We will not measure the cells, but make note of any differences you see.

Collecting White Blood Cell Counts

1.   At your team microscopes, have each teammate focus on one of the other blood samples: mononucleosis, lymphatic leukemia, and myeloblastic leukemia. Have your image focused with the 40x objective in place. Count the number of white blood cells (wbc) in the field of view. There are handheld counters at the team benches to aid in counting if the number is large.

2.   Shift the slide to another spot and again count the number of wbc in the field of view. Shift the slide again and count a third field. Do not add the values together, but keep track of each count.

3.   Have one teammate do the same for the human blood smear (healthy sample).

4.   Organize your team's data in your own observation notebook in a table similar to the example below. Enter into the SPSS spreadsheet. For four patients, your team will add 12 rows to the data file.

**Table 4.** Example white blood cell count data

section	Team	diagnosis*	count
122	4	1	2
122	4	1	0
122	4	1	3

*Diagnosis: 1=normal (healthy) 2= infectious mononucleosis 3= acute
lymphatic leukemia 4= acute myeloblastic leukemia

Root Data Set
      Once a multicellular organism has developed, does cell division continue? Even in multicellular animals that have attained an adult size, there is still cell division for renewal and repair of tissues. In plants, growth can continue throughout the life of the plant. Cell division occurs in specific regions of the plant. These regions are called meristems. There is a meristematic region near the tip of the root within which cells are actively dividing. Consider a root that is growing from the root tip (Fig. 2).

Figure 2. A root tip at one point in time, then at two later times shown from left to right. The line marks one row of cells that is newly formed in the earliest image. This row of cells is much farther away from the tip when the root is examined at a later time point.
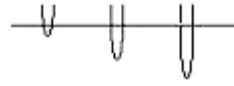
This means that the cells in the root tip are very young cells; they may have only just formed by division of meristem cells. The cells farther up the tip are actually older, by hours, days or weeks, depending on the rate of root growth. In the sections farther up the root, cells may have taken on a more specialized shape and function as they matured.

Collecting Root Cell Measurements

Calibration of the ocular micrometer
1. At one of your team microscopes, focus on any sample. Have all teammates notice the scale from 0 to 100 that appears over the image (Fig. 3A). This is the ocular micrometer, which is a ruler that is in the right eyepiece of the microscope. Rotate the eyepiece and see that it rotates. We will use this scale to measure cells. Size measurements such as 10 units or 22.5 units are relative sizes that you should record as ocular units (o.u.).
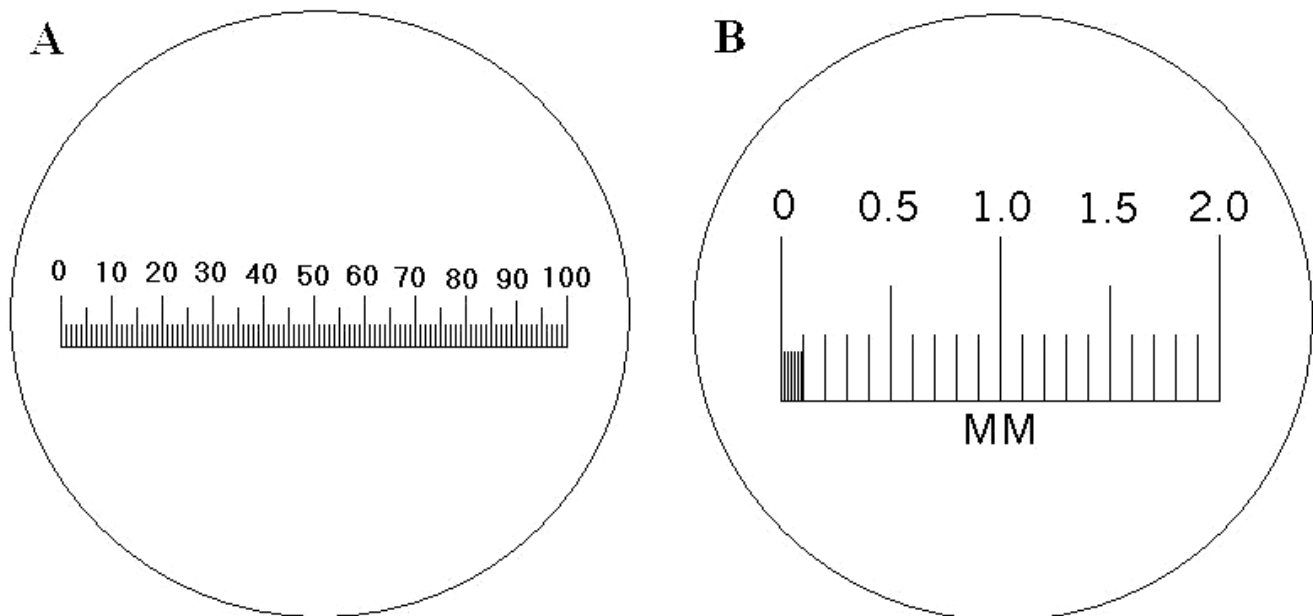
Figure 3. A. view of the ocular micrometer   B. stage micrometer viewed at low power

2. Find the stage micrometer in your team's slide box and focus in on this at the lowest power. The stage micrometer is an actual 2 mm ruler mounted on a slide (Fig. 3B). You should be able to see the whole 2 mm, marked off in 0.1 mm. The 0, 0.5, 1.0, 1.5, and 2.0 mm marks are labeled.

3. The procedure of finding the number of relative units that equal a known quantity is called 'calibration." Calibrate the ocular micrometer at this magnification. To do this, align the zeros of the two scales (Fig. 4). If the ocular micrometer is at an angle, rotate the ocular piece until it is parallel to the stage micrometer's scale. Choose the largest distance in mm you can see and then read the number of ocular units that is equal to. Record your value in Table 6 on the next page. Table 5 below shows an example table with the values for the calibration example (Fig. 4).

Figure 4. Appearance of the two scales during calibration. In this example, 2.00 mm is equal to 86.7 ocular units. The value 86.7 o.u. was recorded because the 2.0 mm line is in between the 86 and 87 marks on the ocular micrometer, but closer to 87 than halfway.
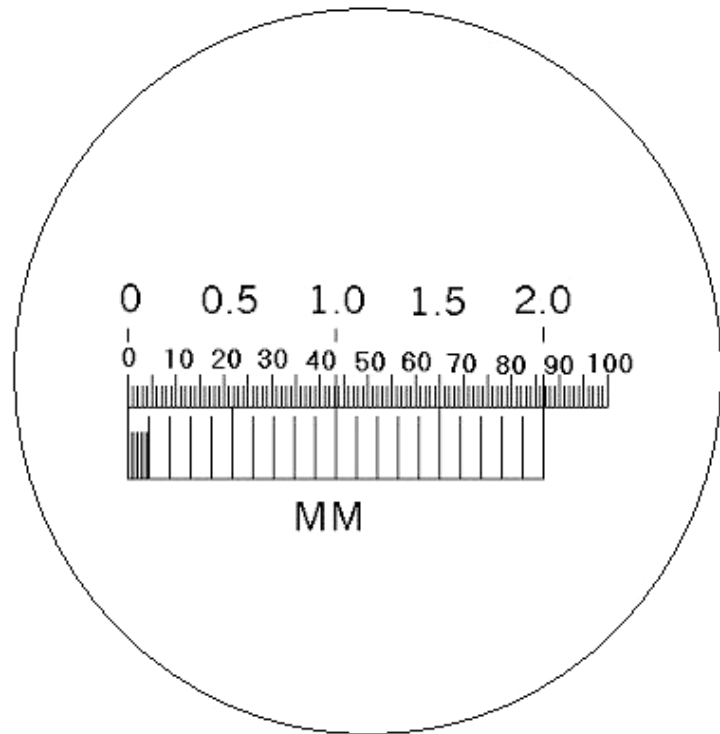
Table 5. Calibration recorded for the example shown in Fig. 4.

Total magnification	d Known distance in mm (stage micrometer)	r Equivalent number of ocular units (ocular micrometer)	d/r Conversion Factor (mm/unit)	d/r Conversion Factor (μm/unit)
40x	2.00 mm	86.7 units	0.0231	23.1

4. Calculate a conversion factor (d/r) that can be used to convert ocular units to actual size in mm.

5. Calibrate your team microscope also for 100x and 400x total magnification, using Table 6. These are the magnifications you will use for cell measurements. Most will use 400x, but 100x may be needed from some large onion root tip cells. There is a different conversion factor for each magnification.

Table 6. Calibration record for Microscope # (keep a copy of this info in your obs manual)

Total magnification	d Known distance in mm (stage micrometer)	r Equivalent number of ocular units (ocular micrometer)	d/r Conversion Factor (mm/unit)	d/r Conversion Factor (μm/unit)

Length and width for root tip cells (prepared slide)

6. Focus on the *Allium* (onion) root tip sample (prepared slide) and find the meristem region (actively dividing region, just behind the root cap, near the very tip). This is the region labeled "1" in Fig. 5. The process of cell division is called mitosis and so your prepared onion root slides may be labeled *onion mitosis* because these are sold for viewing the stages of division (you'll do this next semester in Genetics lab).

Figure 5. Two cells (boxes) in the meristem region *(bottom, region 1)* and a few mm farther up the tip *(top, region 2).* For consistency in our measurements, we will define width as the dimension of the cell <u>across the root tip</u> (in the direction of the width of the root). In a similar manner, length is the dimension of the cell <u>down the column</u> of cells (along the length of the root). It is possible that our recorded "width" will be larger than the dimension we have called "length" for some cells.
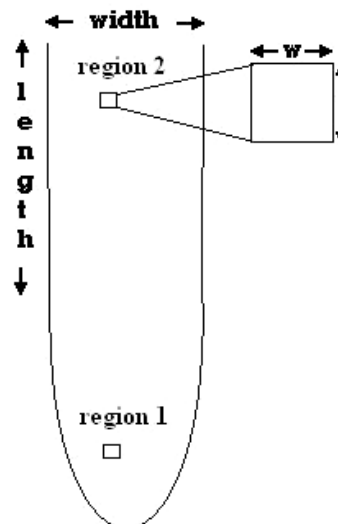
7. Measure the width and length measurements (as shown in Fig. 5) for two cells in the meristem region. These are the youngest cells. Remember to record the magnification you are using when doing measurements, so you later use the correct calibration factor to convert to actual size. Rotate the eyepiece as needed to measure the two different directions using your ocular micrometer.

8. Then measure the length and width of two cells a few mm away from the tip (region 2, Fig. 5)

9. Using the conversion factor for that magnification (Table 6), convert the values to actual size in μm.

    Measurement in o.u. x conversion factor μm/o.u. = actual size in μm

10. Organize your team's data to enter into the SPSS spreadsheet (Table 7). You will add four rows.

**Table 7.** Example root cell length and width data.

section	Team	Region*	width (μm)	length (μm)
122	4	1	16.7	19.0
122	4	2	19.0	102.0

*Region: 1=meristem   2= few mm from tip   3= mature

*Length and width for mature root cells (fresh slide – longitudinal section)*

11. To examine mature cells, we will look at cells farther up the root (40-50 mm from tip). Use a razor blade or scalpel to cut two long roots from one of the scallions provided in the lab. Cut near the top of the root, at the base of the onion bulb.

12. For a longitudinal section, cut across lengthwise along one root near the center (Fig. 6a). Then, make a parallel cut close to it (about 1-1.5 mm away, Fig. 6b). Cut across the root at the base of the cuts to release the small sections (Fig. 6c). Take the center cut (arrow in Fig. 6d). Place on a slide.

Figure 6. A longitudinal section is cut parallel to the long axis of the root. Cut twice near the very center (a, b), then across (c) to release the sections (d). Use the middle section (arrow). On purchased slides, the abbreviation 'ls' is used to indicate longitudinal section.
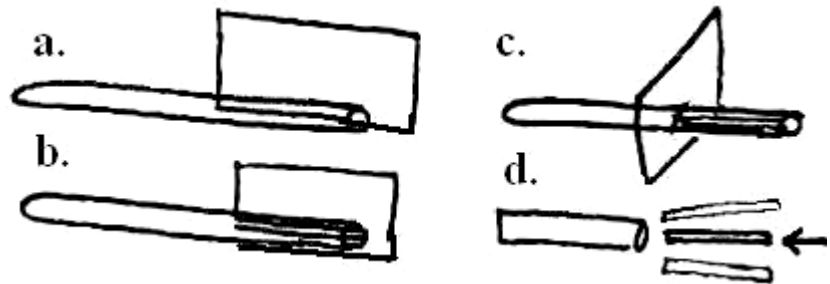
13. Add a drop of the stain provided, wait one minute, then rinse with some distilled water, blotting the excess water with a lint-free wipe. Place a coverslip over the section.

14. Focus on your sample at 400x. Look for distinct cells. Most cells will have a rectangular shape and contain a round or oval darkly-stained nucleus. These cells are ground tissue cells (Fig. 7a). Look around for some cells in the middle of the section that may be stained bluish and have a somewhat striped appearance (Fig. 7b) these are xylem cells. These cells and some of the cells around them are part of the vascular tissue of the root. Notice how in this region of the root not all of the cells look the same. Do they have different sizes and shapes?

Figure 7. Ground tissue cells (a) and xylem cells (b) in the longitudinal root sections.
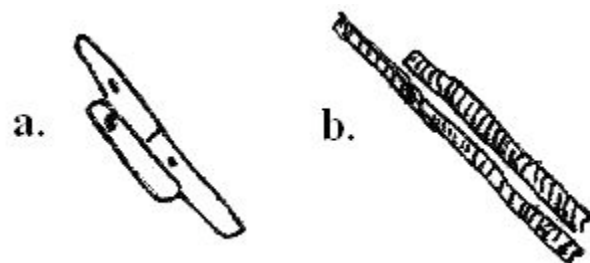
15. Measure the width (shortest dimension) and length (longest dimension) of two ground tissue cells.

16. Convert to actual size in micrometers using your conversion factor (Table 6). Enter your values into the SPSS file (Table 7), indicating these cells as being from region 3 (= mature).

Diameter for mature root cells of different types (fresh slide- cross section)

17. Using your 2nd long scallion root, cut a cross section in the region 40 – 50 mm from the tip (Fig. 8). For the **cross section**, cut across the root, perpendicular to the long axis of the root. Cut twice making a small round section (Fig. 8). Try to make the section as thin as you can while keeping it an intact circle – about 1-2 mm. Place on a clean microscope slide.

Figure 8. A cross section is cut perpendicular to the long axis of the root. Cut twice to make thin cross section. The abbreviation 'cs' is used to indicate cross-section.
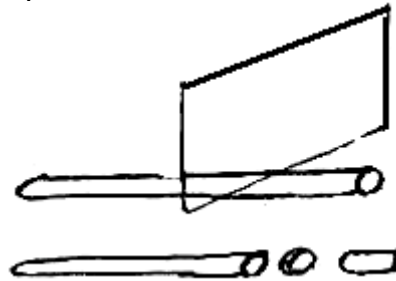
18. Stain the cross section as in step 13. Add a coverslip.

19. When focusing on the sample, take care when swinging the high power objective into place. Your section may be too thick. If it is, examine and make your measurements at 100x (10x objective).

20. Examine your cross section. It will have a central region as shown in Fig. 9. This is the central vascular cylinder, with cells involved in transport of water, nutrients, and sugars throughout the root. Find some large cells with round cross sections and thick walls, identified as xylem (Fig. 9b). These are the cells you saw in the other section having a spiral of cell wall thickenings such that they looked striped. There are small cross-sectional cells between the xylem that are phloem cells (Fig. 9c). Notice the outer ring of cells; this layer is called the endodermis (Fig. 9a). *Inside* this ring is the vascular cylinder. The endodermal cells are actually specialized ground tissue cells, but we will consider them separately from those outside this ring. All the cells outside the center vascular cylinder, except for the outermost layer of the root (epidermis), are ground tissue cells (Fig. 10d).

21. Have your team measure the diameter of two xylem cells, two cells in the endodermis layer, and two ground tissue cells. Record values in ocular units, noting which magnification you used.

Figure 9. Central region of a scallion root cross section. Not all cells are shown.
a. endodermis b. xylem c. phloem
d. ground tissue
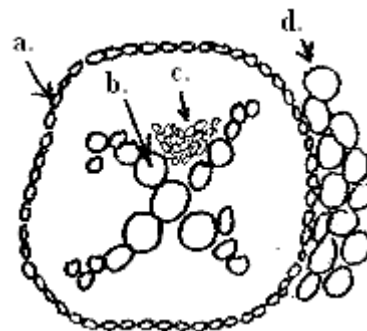
22. Using the conversion factor for that magnification (Table 6), convert the values to actual size in μm.

23. Enter your values into the SPSS file for mature root cell diameters (Table 8).

Table 8. Example mature root cell diameter data.**

section	Team	Celltype*	diameter (μm)
101	5	1	86.0
101	5	2	20.0

*Celltype: 1= xylem   2= endodermis   3= ground tissue
**Note: an additional column (species) may be added if some prepared
cross sections from other species are measured in some lab sections.

24. This was the last cell measurement needed for our data sets. Your team is now free to use their extra lab time to view live protists, visit the microscopy display, or learn the domains/kingdoms (next section).

Types of Microscopy Display
     Do you know the difference between TEM and SEM? Can proteins be seen with microscopy? Are there different types of light microscopy? Look at the examples and information on display.

Domains & Eukaryotic Kingdoms
     At your team bench is a diagram showing the classification of living organisms. Review this information and talk with your teammates about the different types of cells and organisms.
- Do you know which groups have a nuclei in their cells?
- Which groups have mitochondria in their cells?
- Are chloroplasts only found in the cells of plants (PLANTAE)?
- Do you know which groups have cells with cell walls?
- What domain/kingdom are you in? What about onion?

Live Protists
     Bacteria are not the only unicellular organisms. Don't forget to take a look at some of the live protists available during our second week of lab. Make some slides and watch them move! These are in the Kingdom Protista.

Materials

Egg Data set

- M, L, XL eggs (from grocery store) - 2 of each egg type for a team of 3
- Egg cartons cut in half for each team to have 6 eggs
- Egg carton sectioned to 1-egg pieces to use as egg weigh boats

- Calipers (1 per team)
- Electronic balances (1 per team or a few in the classroom, to nearest 0.1 adequate)

Micrograph Data Set

- Micrographs of bacteria, mitochondria, nuclei (for each team: two of each subject -- one with a scale bar and one with magnification shown). Bacteria micrographs should be distributed so shape groups (round, rod, spiral) are represented equally.

- Rulers
- Calculators (available for students who did not bring calculator to first class)

Onion Data Set

- Onion root tip slides (those purchased for mitosis labs work well)
- Scallions (from grocery store, purchase fresh and look for long roots) or purchased mature root slides (cross section & longitudinal section)

- Student microscopes with ocular micrometers (1-3 per team)
- Stage micrometer (1-3 per team) Wards 94 W 9910 is the type pictured in the figures.
- Cutting board and scalpel or razor blade, nuclear stain (such as Azure A) if using fresh roots

Blood Data set

- The following blood slides are available from Wards (Rochester NY, www.wardsci.com):
- Human blood smear slides (1 per team) Wards 93 W 6541
- Iron deficiency anemia blood smear slide (1 per team). Wards 93 W 9033
- Sickle cell anemia slides for examination (1 per team or 2 per class, *optional)* Wards 93 W 8120
- Pernicious anemia slides for examination (1 per team or 2 per class, *optional)* Wards 93 W 9058
- Infectious mononucleosis blood smear slide (1 per team) Wards 93 W 9052
- Acute lymphatic leukemia blood smear slide (1 per team) Wards 93 W 9040

- Acute myeloblastic (monocytic) leukemia blood smear slide (1 per team) Wards 93 W 9048
- Station with microscope, digital camera, computer with measuring software (if available) <u>or</u>
- Student microscopes with ocular micrometers (1-3 per team). Wbc counts can be done with microscopes lacking ocular micrometers.
- Pictures of different white blood cell types
- Instructions for use of measuring software

Data Entry / Statistical Software

- computers for data entry (entry into SPSS directly or into EXCEL, minimum 2 per lab room)
- Data files (SPSS or EXCEL) set up for each data set with variables defined
- Projector to display computer entry during egg data set (used as demonstration/practice)

- Availability of computer classroom for introduction to SPSS or other statistical package
- University network license for statistical package for student access for data analysis (The textbook for our MAT 1281 Applied Statistics included a CD with SPSS student version).

For Practice With Microscopes/Introduction to Domains& Kingdoms, Microscopy
(optional additional material)
- Letter e slide or other practice slide (tiny letter slips under unbreakable coverslips work well)
- Example protist, animal, plant slides such as amoeba, human kidney, privet leaf
- List or diagram of the Domains and eukaryotic Kingdoms

- Information on different types of microscopy. We use a 3-fold display board with the diagrams of compound, TEM, SEM microscopy (showing light or electron beam path) and info about fluorescent, phase contrast, and freeze fracture microscopy from their class textbook.
- Live protist samples. We have preferred *Blepharisma* (Wards 87 W 1000), *Stentor coeruleus* (Wards 87 W 1370), and Chaos (Wards 87 W 0700) because they are large and colored to make it easier to find them under the microscope. Purchase some *Paramecium* to feed *Stentor* and *Chaos*. Ciliate and flagellate mixture cultures have also worked well.

Notes for the Instructor

1. **Overall timetable of lab exercise & necessary coordinating activities**
    First two lab sessions, 6 hour total (times approximate):
    - 1 hr for introduction to data sets/practice egg data set (see note 2 below)
    - 1 hr for introduction to units, use of microscope, time to practice using microscope
    - 3 hr student teams collect data for the 3 main data sets (time split between two lab sessions)
    - 1 hr introduction to the statistical package (at computer classroom, using tutorial)

    After 1st week of lab
    - Coordinating instructor combines all egg data into large data set
    - Any additional egg measurements made by TA (see note 3 below)
    - Data set made available on campus network public drive for Applied Statistics classes

    After 2nd week of lab
    - Coordinating instructor combines all section data into large files for three main data sets
    - Coordinating instructor sorts by section & prints for section instructors (see note 4 below)

    3rd week of lab (Another lab experiment is conducted this week)
    - Instructor can ask teams with missing or problematic data to check/add their entries

    After 3rd week of lab
    - Coordinating instructor finalizes large files for three main data sets
    - Data sets made available on campus network public drive for students

    Student work outside of class time
    - Project in Applied Statistics class using biology lab data sets (see note 5 below)
    - Analysis of data set - using descriptive statistics (ave, std dev, histograms, box plots,…)
      A handout with tips on how to do this is provided
    - Student teams meet to prepare a short presentation for biology lab
      A handout with information about their team's "biological focus" is provided
    During a later lab session
    - Student teams give a short presentations (about 15 min) showing the results of their analysis.
      Each team has a different focus to avoid overlap from team to team

2. Practice Data Set: As a class, the instructor has the students collect egg length, width, and mass (each team of three has 6 eggs to measure). The instructor enters the data into SPSS (or other statistical package) while displaying the entry on a screen so the students can become acquainted with data entry. The instructor shows briefly how, once entered, the statistical package can calculate values such as average, minimum and maximum and produce graphs such as histograms for the data set. This is to orient the students with an overview of data collection, entry, and to show them that obtaining graphs/values from the statistical package will not be difficult. Since this is probably one of their first few days in college, the emphasis here has been to calm them down and make the subsequent data collection less confusing. The egg data set is then combined and made available as one file to the statistics instructors who use it for class examples or projects. One feature of the software SPSS that did need to be shown was the use of value labels that are entered with a drop-down menu (once the cell is selected) or by typing in a number (for example 1=L, 2=XL) when entering data into a file for which the variables were already defined. When not introduced, students tried to type in, for example, the L or XL and became frustrated.

3. Eggs: Fresh eggs were used. In our labs, each egg was numbered using a marker. Let eggs come to room temperature before marking (or measuring) to avoid a wet surface. The size M, L, XL was also written on the egg. Eggs were measured more than once because the same half dozen eggs was used by teams on different lab days. For easier use in the statistics classes, we tried have each egg measured the same number of times (4 times). A variable for replicate number was added to the data file. After compiling the large combined data set, those eggs measured fewer times were identified. A TA measured those eggs to provide the additional measures. Multiple measures of same egg was found to be useful for statistics class topics.

4. Missing data: Instructors can check over their class entries for missing or noticeably erroneous data. Some team data may indeed be in the students' notebook but not entered on the computer. Allowing an additional week to remind these students to enter their data has been helpful. This is also a good time to have students correct for conversion errors that make values off by 1000x or 100x -- these errors are easy to spot in the combined data set. One other error that has been seen is the use of inches for eggs when all other students have used mm. Also, at times, length has been entered in the width column (and vice versa) or "ocular unit" measures entered in the "actual size in um" column. Corrections can be confirmed against team records this 3rd week. If class time during the 3rd week is at a premium, emailing teams with questions is an option that has worked -- students will stop by before or after lecture class and make the corrections if alerted to possible problems. Not all problems need to be corrected, though -- it is a useful exercise for students to have to look for outliers and decide about trimming the data set when working on their statistics projects.

5. Overlap of Statistics and Biology Projects: Students who are concurrently enrolled in the Applied Statistics course have a project using the cell measurement data. Their analysis for biology lab overlaps with that they will have completed for their statistics class. Lab teams are adjusted the first day of lab so that at least one member of each team is enrolled in Applied Statistics. It is useful to make sure students know which of the six projects they are working on for biology lab so that they can work on the same data set for their statistics class. When we let teams choose their project, they often could not remember which to tell their statistics instructor. So, now we assign projects by team number and provide a room layout so there should be no question which project they have been assigned (as long as they know where they sit in bio lab).

6. Micrographs: For this lab, we used micrographs prepared by students in our Electron Microscopy class supplemented by other micrographs. There have been some difficulties from the point of view of the statistics class projects with this data set, because of the smaller sample sizes. For the bacteria, there are only 8 micrographs for each shape group. This makes this data set different from the other sets that have more than a hundred cells measured; this is not desirable for the statistics class projects. For the biology lab presentations, however, the data set has been useful. The range of sizes of the different shape categories does differ and makes comparison interesting. Micrographs with bacterial populations may help resolve this issue.

7. Scallion Sections: Variable results have been obtained with different batches of scallions - some have made sectioning easy whereas, with other batches, most students could not obtain a useful section. It is helpful to have some prepared slides on hand to use if difficulties arise. Having some measurements of onion and also of another species (such as buttercup for which prepared slides are available) gives students an additional comparison when analyzing data.

8. White Blood Cell Identification: The lab exercise has a dichotomous key for distinguishing the different types of white blood cells and we provided images of each type for comparison. Even so, misidentification was common. It would be helpful to have a TA help the students when they are at this point or to review the identifications the students have made.

9. Getting teams on the right track: When starting a 1 hr session of measuring it is useful to get the students working on micrographs started in the right direction and then spend more time working with the students with the onion measurements. The calibration of the ocular micrometer is difficult for students. Tables right next to one another are assigned in the schedule to work on the root data simultaneously to allow one instructor to explain the calibration to the two teams at the same time. It is necessary to check on teams working on the blood data to make sure they also do not get stuck on the calibration step. Because of the limited number of digital camera stations (we had 2 per class) it is necessary for teams to wrap up their blood measurements in about a 1 hour time period. Teams can get stuck and need assistance periodically in order to be able to finish in the 1 hour period; they should not be left on their own for the full hour.

10. Keeping track of all teams need to finish: It was very helpful to put on the blackboard a checklist of what measurements they needed to collect -- such as "3 red blood cell diameters, 3 white blood cell diameters, 3 anemic red blood cell diameters". This helped some samples (such as the iron-deficiency anemia) from being forgotten. It also helped teams see what they have accomplished/what they had left to finish to keep them from being overwhelmed. Actually, each team only measures a few of each type of cell.

11. Independent work: In the 2nd lab session, teams tend to work more independently. They have started their measurements and have had a week to calm down (no longer their first week of college) and hopefully read over the lab. This 2nd session was designed this way because we miss our 2nd lab session on Monday due to Labor Day holiday. We ask our Monday teams to come in to make up the lab, offering several options for times they can do this. We make sure an instructor is there when they make up, but overall they are able to work more independently in this second week.

Acknowledgements

About the Authors

Denise Marie Ratterman received her Ph.D. from University of Wisconsin-Madison. She has been teaching and coordinating the freshmen biology labs (Bio I: Cells and Bio II: Genetics) at SJU since 1994. A freshman advisor and a resident faculty living in the freshman "Science Hall" (since 1996) in addition to teaching the freshmen, Dr. Ratterman has been working to make the freshman lab experience both academically-demanding and supportive.

James Watrous received his Ph.D. from Georgetown University, and has been teaching at SJU since 1972. A former chair of the department, Dr. Watrous has been instrumental in bringing biometry and computer modeling into the biology curriculum for both undergraduate and graduate students. In addition to upper division courses, he currently teaches several sections of the Bio I: Cells lab, working to incorporate the new IBASE initiative of integrating statistics education and introductory biology lab for first semester students.

Deborah Lurie received her Ph.D. from Temple University, and has been teaching at SJU since 1997. As a biostatistician, she has done extensive collaborative research in reproductive endocrinology, in the area of *in vitro* fertilization. Dr. Lurie has worked to develop and teach an applied statistics course designed for first year biology undergraduates. She has coordinated the integration of the statistic course with the biology lab among the multiple sections/instructors for the statistics course.

Appendix: SPSS tutorial, schedule, and handout information

The SPSS tutorial used with this lab exercise, course schedules for both Bio I lab and the Applied Statistics course, and other handouts used to assist students when working on their projects are available in document and pdf format at the IBASE website at www.sju.edu/cas/ibase . The SPSS tutorial will be updated for new versions of SPSS as necessary. The dichotomous key used for the white blood cell identification and problems to avoid images will also be made available at the site.