Laboratory 2
Introduction to Microscopy

I. Introduction

All living organisms are composed of cells. While some organisms are unicellular and consist of a single, independent cell, others are multicellular and consist of thousands or millions of physically-connected, interacting cells. In a multicellular organism, these cells are often organized into tissues, which in turn are assembled into organs and organ systems. Each tissue of a plant or animal is composed of different cell types, which vary in structure and function. A variety of techniques are currently available for studying the cells of a particular tissue by both light and electron microscopy. The purpose of this laboratory session is to introduce the basic techniques of light microscopy.

As part of this lab, you will:

- learn to set up and use a binocular light microscope correctly
- calibrate an ocular micrometer or reticle so that quantitative measurements of cells can be made
- use bright-field, dark-field, and phase contrast optics to visualize human cheek cells
- use bright-field optics to make similar observations of photosynthetic cells from *Elodea*
- carry out a series of basic calculations related to cell sizes and volumes.

As you carry out the experiments, make notes, record your observations, and write down your measurements and calculations. For this lab session, you will need to make drawings of the cells that you see on a Data Sheet. You do not need to include every cell in the visual field, but you should include some representative cells drawn to the correct scale. Be sure to indicate the total magnification next to each drawing. This experiment is adapted from one originally described in Helms, D. R., Helms, C. W., Kosinski, R. J., and Cummings, J. R. (1998) **Biology in the Laboratory**, 3/e. W. H. Freeman and Company, New York, NY.

II. Pre-Lab Preparation

Read the Introduction, Background Information, and Experimental Procedures for this laboratory session. Also, read the sections of your Cell Biology textbook which deal with light microscopy and basic cell structure. After preparing for the lab, you should be able to answer the following questions.

A. What is the difference between magnification and resolution?

B. What is the maximum magnification and resolution normally achievable with a compound light microscope?

C. How does the wavelength of light affect the resolution of a microscope?

D. Why is immersion oil used with certain high magnification objectives?

E. If you examine a sample with a 40X objective lens and a 10X ocular lens, what is the total magnification?

F. What is the purpose of the condenser lens?

G. What is the difference between a positive stain and a negative stain?

H. What is the advantage of using a microscope with phase-contrast optics compared to a microscope with bright-field optics?

I. What is the advantage of using a microscope with dark-field optics compared to a microscope with bright-field optics?

J. What is the purpose of calibrating an ocular micrometer with a stage micrometer?

K. Why is a cover slip (cover glass) placed on top of a "wet-mount" preparation?

L. Why is a stain like methylene blue usually used to visualize cheek cells?

M. Why is methylene blue usually not necessary to visualize plant leaf cells?

III. Background Information

A microscope is an instrument that magnifies an image of an object so that it is visible to the human eye. There are several different types of microscopes. Light microscopes use visible light as a source of illumination and result in an image that can be seen directly with the human eye or recorded on photographic film or a digital camera. Fluorescence microscopes use ultraviolet (UV) light as a source of illumination and result in an image that also can be seen either directly with the human eye or recorded on film or a digital camera. Electron microscopes use beams of electrons as a source of illumination; they result in an image that can be detected only on a fluorescent screen, photographic film, or digital camera. Each type of microscope has specific limitations and uses.

A. Light Microscope Components

The diagram shown in Figure 2.1 illustrates the components of typical bright-field light microscope and the path of light through the instrument. Light from a light source or lamp is focused on the sample by a condenser lens. The condenser lens usually contains a diaphragm that allows the intensity of the light to be increased or decreased. The sample is usually mounted on a slide, which can be moved around on a mechanical stage. The light that passes through the sample then enters an objective lens. Most microscopes have several objective lenses, which are mounted on a revolving nosepiece. The light then passes through a second ocular lens and enters the eye or camera system. To bring the image of an object into focus, the stage and the sample (or sometimes the objectives) are moved up and down using a coarse focusing knob and a fine focusing knob on the side of the instrument.

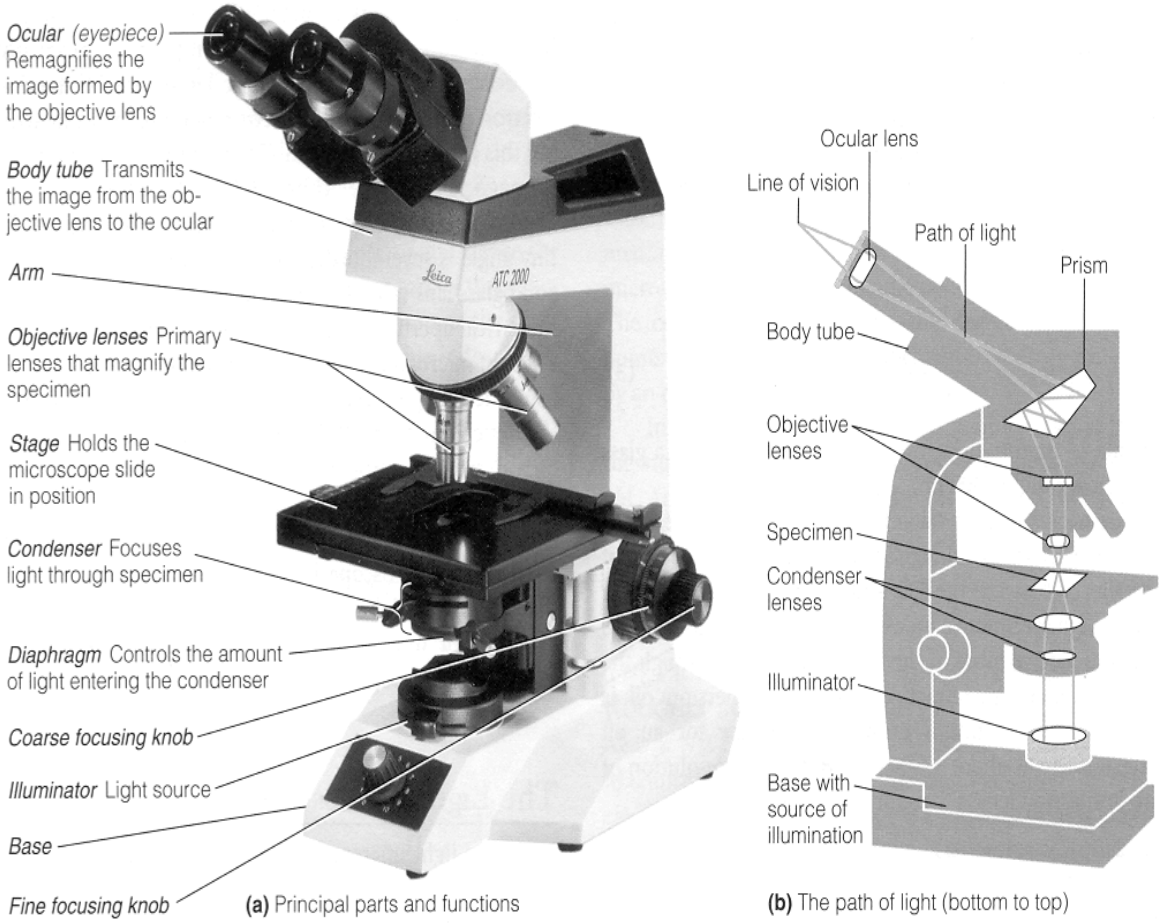


Figure 2.1. Compound Light Microscope. (a) A compound light microscope. (b) The path of light through the compound microscope.

B. Magnification and Resolution

Two key terms are used to describe any microscope. The first term is magnification, which refers to the number of times the image of an object is enlarged by the microscope. The total magnification in a microscope is the product of the magnifications of each of the individual lenses. Compound light or fluorescence microscopes usually have two lenses (an objective lens and an ocular lens) and produce total magnifications between 40X and 1000X (Figure 3.1). If you use a microscope with a 40X objective lens and a 10X ocular lens, the total magnification is (40X x 10X = 400X). The magnification of a light microscope is limited in part by the ability of the manufacturer to prepare lenses that transmit light with minimal optical distortion. Most objective lenses are actually assembled from several parts. By contrast, electron microscopes have at least three magnetic lenses and produce magnifications between 1000X and 100,000X.

The second term is resolution or resolving power, which refers to the ability of the microscope to produce an image in which two objects or structures are seen as separate entities. The resolving power (**R**) or limit of resolution (**d**) of a microscope is expressed as the distance (in m or nm) between two resolvable points. **The smaller the value of R or d, the better is the resolution or resolving power of the microscope.**

R or **d** can be calculated using the expression:

$$\mathbf{R} = \frac{0.61\,\lambda}{\mathbf{n}\sin(1/2\,\boldsymbol{\theta})}$$

where **λ** (lambda) is the wavelength of light passing through the instrument and the sample, **n** is the refractive index of the medium between the sample and the objective lens, and **θ** (theta) is the angular aperture of the light cone that passes into the objective lens. The angle 1/2 **θ** is also sometimes called **α**. The combined term **n** sin (1/2 **θ**) is called the numerical aperture and is a property of each objective lens.

Light rays from the condenser are focused on the sample and then cross as they proceed on to the objective lens. Note the angle (theta) (Figure 2.2).

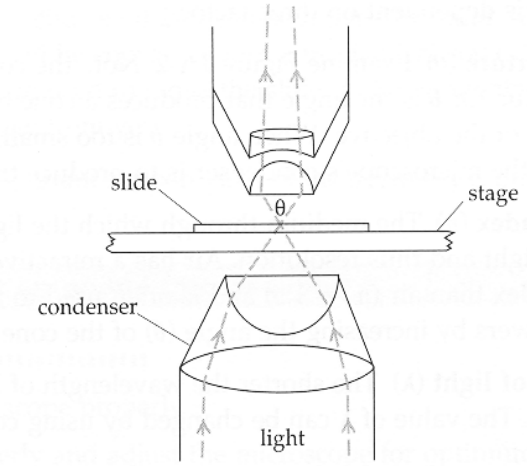


Figure 2.2. The microscope condenser focuses light onto the specimen on the microscope stage. Observe the point at which the dotted lines cross.

Given the equation shown above, it follows that the resolving power of a light microscope can be increased (**d** gets smaller) either by decreasing the value of the numerator (0.61 **λ**) or by increasing the value of the denominator [**n** sin (1/2 **θ**)]. By using shorter wavelengths of light, the resolution of a light microscope can be increased. Many microscopes have a blue light filter in the base of the instrument between the light source and the condenser to improve the resolution. On the other hand, the numerical aperture of lens can be increased either by increasing **n** (the refractive index) or by increasing **θ**. As light moves through the glass of the slide and enters the air space between the sample and the objective lens, it is scattered by refraction. For maximum resolution, the objective can be immersed in a special oil that has the same refractive index as glass. This minimizes the scattering of light (Figure 2.3).

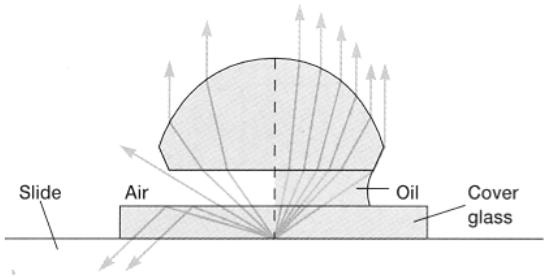


Figure 2.3. The Oil Immersion Objective. An oil immersion objective operating in air and with immersion oil.

The numerical aperture also can be increased by using an objective lens that will produce a focused image when it is very close to the sample. The working distance between the object and the sample usually gets smaller as the magnification of the objective increases (Figure 2.4). As this occurs, the aperture angle **θ** gets larger and so does the sin **θ**. This increases the term **n sin (1/2 θ)** and so leads to an improvement in the resolving power of the microscope.

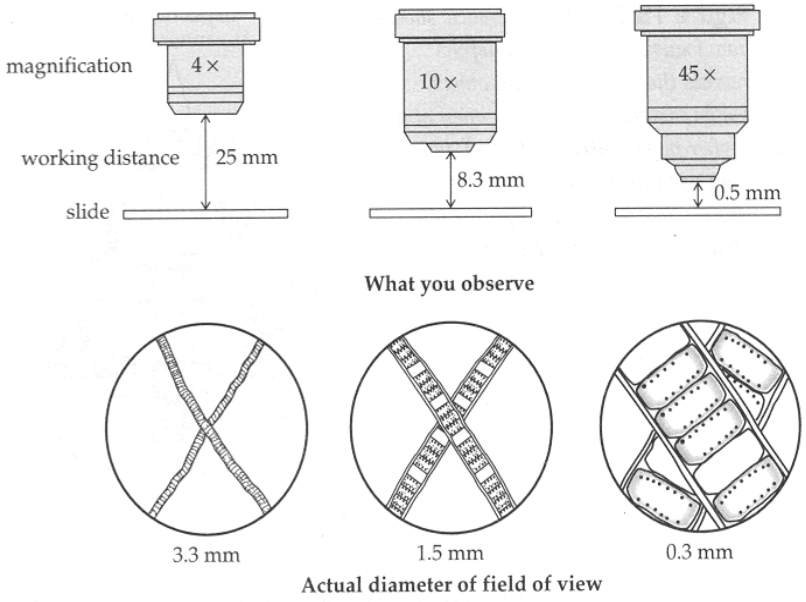


Figure 2.4. The field of view and the working distance change with magnification. (When each of these magnifications is used with a 10X ocular, the magnification is multiplied by 10.)

The properties of the four different objective lenses that are commonly found on a laboratory microscope. Note that immersion oil is used only with the highest power (100X) objective lens (Table 2.1).

Table 2.1. The Properties of Microscope Objectives

	Objective			
Property	**Scanning**	**Low Power**	**High Power**	**Oil Immersion**
Magnification	4×	10×	40–45×	90–100×
Numerical aperture	0.10	0.25	0.55–0.65	1.25–1.4
Approximate focal length (*f*)	40 mm	16 mm	4 mm	1.8–2.0 mm
Working distance	17–20 mm	4–8 mm	0.5–0.7 mm	0.1 mm
Approximate resolving power with light of 450 nm (blue light)	2.3 µm	0.9 µm	0.35 µm	0.18 µm

C. Staining

Some cells have an intrinsic color and so can be easily seen in a light microscope against a bright background of light. For example, photosynthetic organisms like algae contain a green pigment called chlorophyll which makes the cells appear bright green. Most other cells, however, are transparent and this makes them much harder to see them against the bright background. These transparent objects can be visualized by the use of stains. A positive stain is a dye that binds to certain molecules inside a cell or on its surface and so makes the cells colored. The cells then stand out against a light background. Methylene blue and crystal violet are commonly-used examples of positive stains. Some positive stains are acidic compounds that contain -COOH or -OH groups. These groups may undergo ionization (deprotonation) to form negatively-charged groups, which then can bind to positively-charged molecules such as proteins found in the cells. Other positive stains are basic compounds that contain $-NH_2$ groups. These groups may undergo protonation to form positively-charged groups, which then can bind to negatively-charged molecules such as DNAs and RNAs in cells. Some positive stains are nonspecific but others bind more specifically to particular types of biomolecules. A few positive stains are so-called "vital dyes" that can stain cells without killing them. However, most stains are dissolved in organic solvents like ethanol and/or acids and bases and so staining the cells usually kills them. By contrast, a negative stain is a dark insoluble compound such as India Ink or Nigrosin. When transparent cells are suspended in a thin layer of this type of stain, they appear as light objects against a darker background.

D. Dark-Field, Phase-Contrast and Differential-Interference Contrast Microscopes

The basic optical system of a bright-field light microscope can be modified to produce dark-field, phase-contrast, and differential-interference contrast effects. Microscopes with these features make it possible to see live cells that are transparent without staining or killing them.

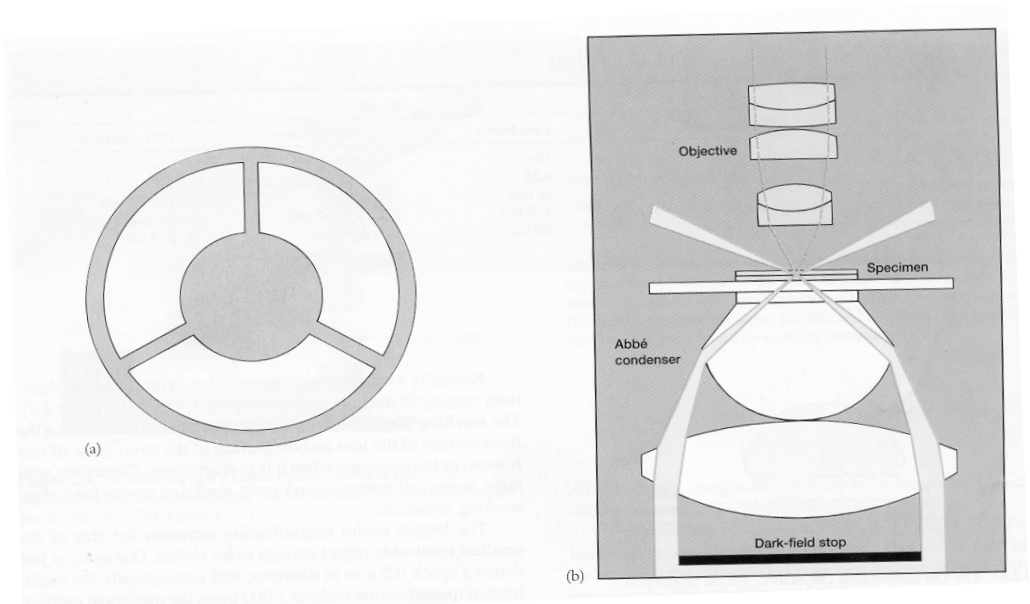


Figure 2.5. Dark-Field Microscopy. The simplest way to convert a microscope to dark-field is to place (a) a dark field stop underneath (b) The condenser lens system. The condenser then produces a hollow cone of light so that only light entering the objective comes from the specimen.

A dark-field stop or block is introduced into the light path so that the sample is illuminated with a cone of light (Figure 2.5). The unreflected and unrefracted rays of light do not enter the objective lens and so the background appears dark. Only light that has been reflected or refracted by the sample can enter the objective lens and so forms an image of the specimen. The result is an image of bright unstained cells against a dark background.

In the case of phase-contrast microscopy, an image is produced as a result of differences in the thickness or the refractive index of the cells in the sample (Figure 2.6a). This requires a special phase plate with an annular diaphragm in the condenser, which is matched to phase plate in the objective lens. The cells appear as dark objects against a light background. In the case of differential-interference contrast microscopy, polarization filters exploit differences in thickness or refractive indices and create a three-dimensional image of the cells (Figure 2.6b). Other modifications of the light microscope allow the creation of images from fluorescent light. A series of pictures as seen with different types of light microscopes are shown in Figure 2.7. You will make similar observations of your own cheek cells as part of this lab.

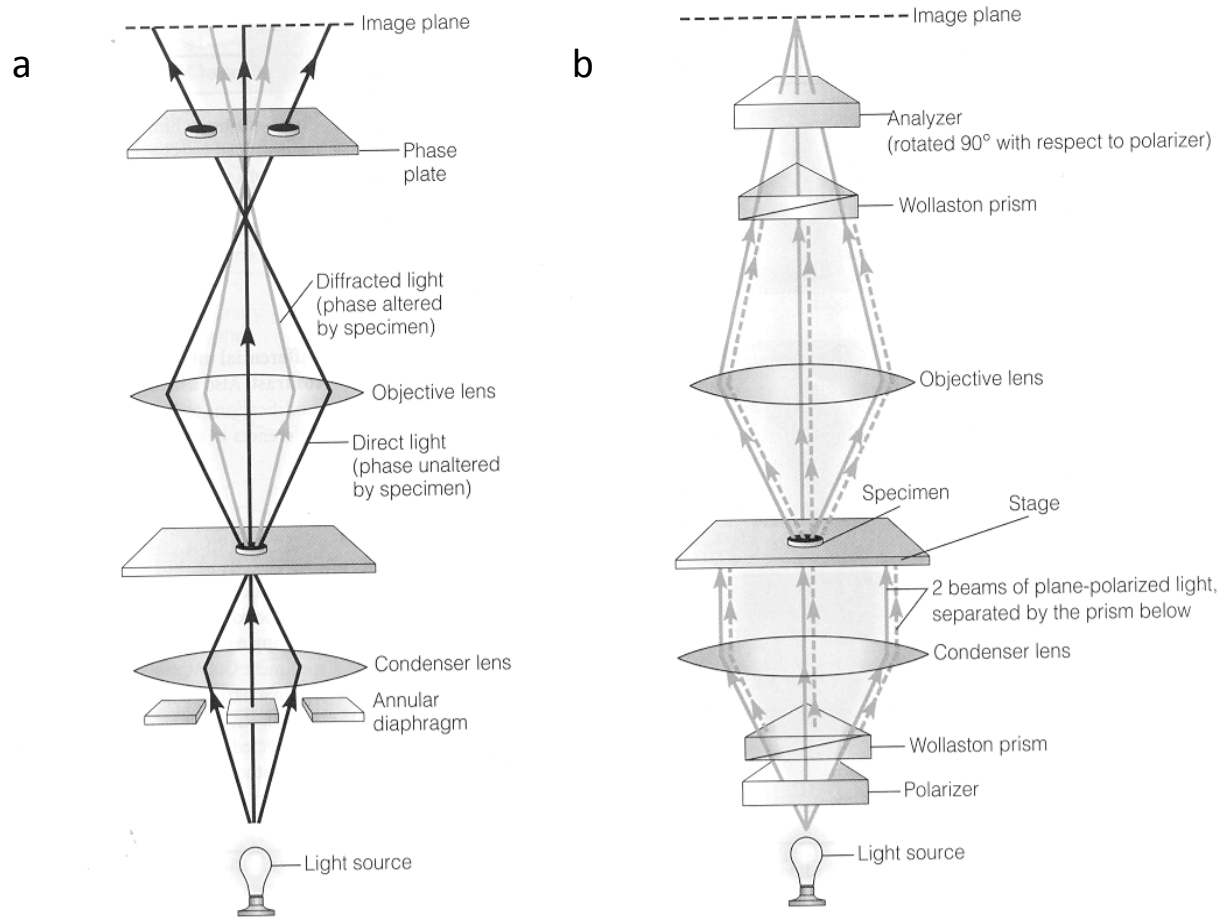


Figure 2.6. Optics and Configuration of the (a) Phase-Contrast and (b) Differential Interference Contrast (DIC) Microscopes

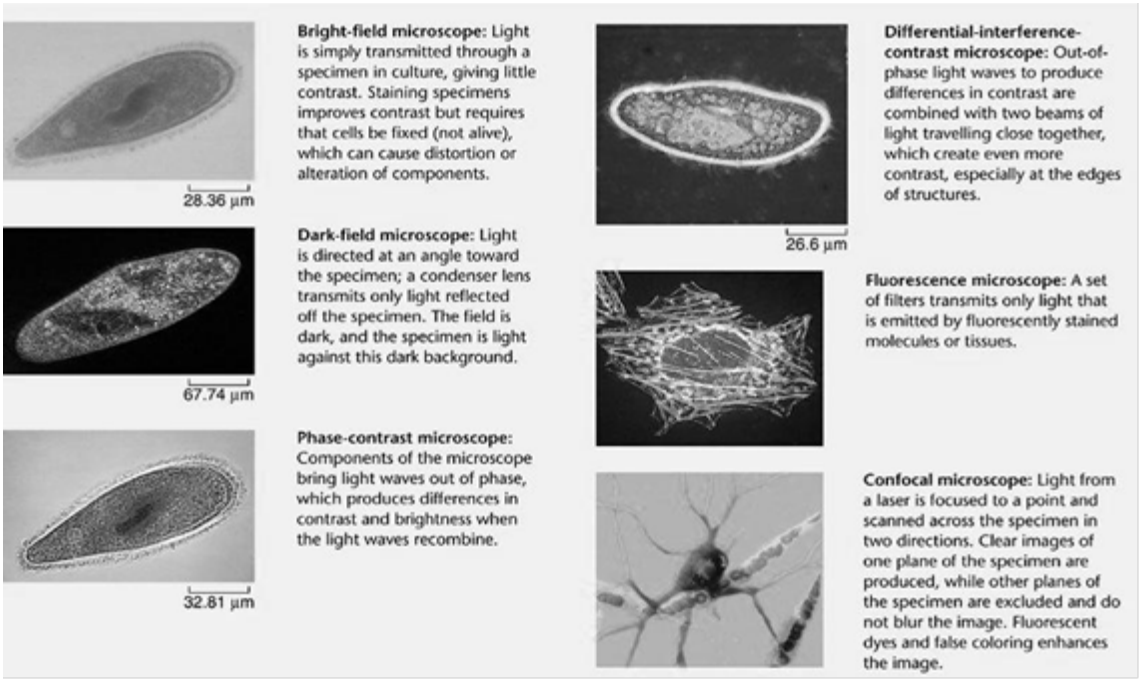


Figure 2.7. Types of Light Microscopy: A Comparison

E. Calibration of the Ocular Micrometer

As part of this lab you will need to calibrate a scale (an ocular micrometer or reticle) in the right eyepiece of the microscope so that it can be used to measure the dimensions of different types of cells. The ocular micrometer scale itself has no inherent units, and because different objectives produce images with different degrees of magnification, the meaning of its intervals varies from one objective to the next. Figure 2.8 shows a slide with some red blood cells as seen through an ocular lens fitted with an ocular micrometer. In this case, the scale simply goes from 1 to 10, and each interval is divided into 10 smaller units.

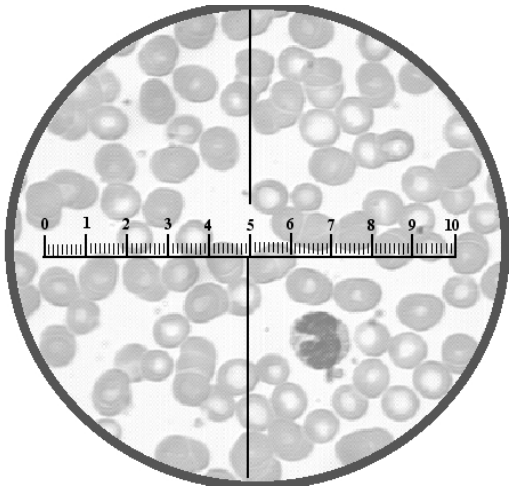


Figure 2.8. View through the objective lens of a microscope with an ocular micrometer.

Because the ocular micrometer or reticle scale has no inherent units, it is necessary to calibrate it using a stage micrometer. A stage micrometer is a special microscope slide with a ruler etched on its surface, which has units of millimeters (mm) and micrometers (μm). To calibrate the reticle, you will line up the stage micrometer with the ocular micrometer and count the number of units or divisions on the ocular micrometer that corresponds to a particular distance in millimeters or micrometers on the stage micrometer (Figure 2.9). **The number of ocular units per millimeter or micrometer will change as the magnification changes.**

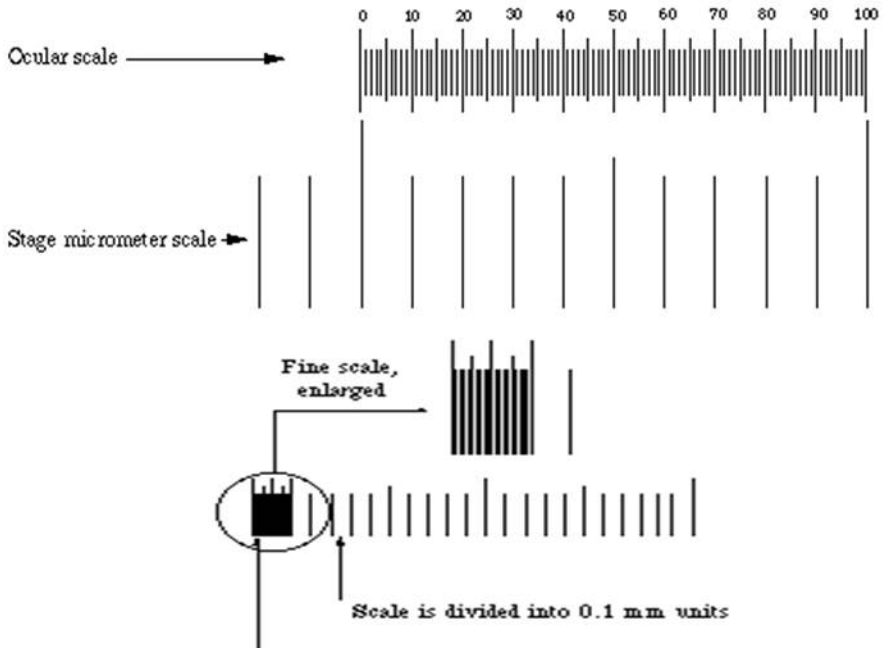


Figure 2.9. (a) The view through the microscope if the stage micrometer has been aligned with the ocular micrometer and focused correctly. (b) The scale on a sample stage micrometer.

The reticles in our microscopes have an ocular scale with a total of 100 units; each major 10-unit division is divided into smaller 1-unit divisions. The stage micrometers you will be using have a scale that is divided into 1 mm units (remember that 1 mm = 1000 µm). These large units are then divided into 0.1 mm (100 µm) units. At one end, the scale is further divided into still smaller 0.02 mm (20 µm) units.

Suppose that using the 10X objective lens and a 10X ocular lens to give a total magnification of 100X, you line up the stage micrometer scale and the reticle or ocular micrometer scale as shown on Figure 2.9. Notice that there are 10 ocular micrometer units for each 0.1 mm (100 µm) unit on the stage micrometer. You can calculate a calibration factor for the ocular micrometer at this magnification as follows:

$$\frac{0.1 \text{ mm}}{10 \text{ ocular units}} \times \frac{1000 \text{ µm}}{1 \text{ mm}} = \mathbf{\frac{10 \text{ µm}}{1 \text{ ocular unit}}}$$

What will be the calibration factor if you switch to the 20X objective lens, giving a total magnification of 200X? The image will be twice as large, so each 0.1 mm unit (100 µm) on the stage micrometer will now span 20 units in the ocular micrometer scale. The calibration factor will be:

$$\frac{0.1 \text{ mm}}{20 \text{ ocular units}} \times \frac{1000 \text{ µm}}{1 \text{ mm}} = \mathbf{\frac{5 \text{ µm}}{1 \text{ ocular units}}}$$

With the 40X objective and a total magnification of 400X, the image will be four times as large as it was with the 10X objective lens and each 0.1 mm unit will span 40 units on the ocular micrometer scale. The calibration factor will be:

$$\frac{0.1 \text{ mm}}{40 \text{ ocular units}} \times \frac{1000 \text{ µm}}{1 \text{ mm}} = \mathbf{\frac{2.5 \text{ µm}}{1 \text{ ocular units}}}$$

With the 100X objective and a total magnification of 1000X, the image will be ten times as large as it was with the 10X objective lens and each 0.1 mm unit will span 100 units on the ocular micrometer scale. The calibration factor will be:

$$\frac{0.1\text{ mm}}{100\text{ ocular units}} \times \frac{1000\ \mu\text{m}}{1\text{ mm}} = \mathbf{\frac{1\ \mu m}{1\text{ ocular units}}}$$

Notice that as the magnification increases, the distance represented by each ocular unit becomes progressively smaller.

The scales on the stage and the ocular micrometers rarely line up this precisely. Suppose that a student used a different microscope with a 4X objective and 10X ocular lens and found that there were 42 ocular micrometer divisions per millimeter. What is the distance in micrometers per ocular unit?

The total magnification is 40X. The calibration factor is

$$\frac{1\text{ mm}}{42\text{ ocular units}} \times \frac{1000\ \mu\text{m}}{\text{mm}} = \frac{23.8\ \mu\text{m}}{\text{ocular unit}}$$

Using these values, answer the following problems to test your understanding of the concepts shown above.

Problem 1: At a total magnification of 100X, you measure an item that is 10 ocular units long how long is it in micrometers?

_____

Problem 2: At a total magnification of 400X, You measure the size of the nucleus of a cell and find it is 5 ocular units how large is the nucleus in micrometers?

_____

Problem 3: Notice the pattern in the above calibrations. If the distance in micrometers per ocular unit at 100X is 20 µm/ ocular unit what would it be at 1000X?

_____

Problem 4: Suppose you use this microscope to measure the diameters of five (5) red blood cells using a total magnification of 400X. You obtain values of 12.7, 12.3, 12.5, 12.6, and 12.3 ocular units. What is the average size of the red blood cells in micrometers?

_____

IV. Experimental Procedures

This experiment involves several parts. Each person should use his/her own microscope for the basic observations, but you can then work as a group on the remaining parts of the experiment. Once a person has made a good slide, the other people in the group should look at it. You will need to share the stage micrometers and the microscopes with reticles for the quantitative measurements.

The following is a flowchart for this laboratory session:

Basic Light Microscopic Techniques (Section IVA)
↓
Calibration of the Ocular Micrometer (Section IVB)
↓
Microscopic Observations of Human Cheek Cells (Section IVC)
↓
Microscopic Observations of Elodea Cells (Section IVD)
↓
Calculation of Cell Volumes (Section IVE)

A. Basic Light Microscopic Techniques

The objective of this part of the lab is to learn to use a bright-field light microscope effectively. The microscopes to be used in the course are Leica DME binocular microscopes, an example of which is shown on the next page. They have multiple phase-contrast objective lenses (10X, 20X, 40X, 100X), 10X ocular lenses, a rotating condenser that allows the use of bright-field, dark-field, and phase contrast illumination, and a mechanical stage. The phase-contrast objectives can be used with bright-field, dark-field, or phase contrast illumination without major problems.

1. Obtain a microscope from the cabinet and position it on the bench in front of you. Always use both hands to carry a microscope and set it gently on the table. **Once the microscope is positioned in front of you, DO NOT SLIDE IT on the table.** The head of the microscope should be rotated so that the ocular lenses are pointed towards you and the arm of the microscope is oriented towards the middle of the lab bench.

2. Use Figure 2.1 as a guide and identify all of the major components of your microscope.

3. With lens tissue and the special cleaning solution, carefully clean the two ocular lenses, the objective lenses, the condenser lens, and the light source.

4. To set up the microscope, move the condenser adjustment knob so that the condenser is positioned just under the stage. Set the condenser turret to the bright-field (BF) position. Rotate the nosepiece so that the low power objective lens (10X) is centered and locked into place. Turn on the light source with the power switch and note how the intensity can be varied. Open and close the iris diaphragm as necessary to adjust the illumination. To reduce the light intensity, you should always decrease the power to the lamp or close the iris diaphragm; do not move the condenser out of position. Adjust the distance between the ocular lenses so you can look at slides with both eyes open. Use the coarse focusing knob to move the stage up and down.

5. Obtain a prepared slide with a specimen of a stained animal tissue mounted under a cover slip. The tissue sample in this slide was fixed and embedded in paraffin, sliced into thin sections with a microtome, and stained to make the cells visible. Position the slide on the mechanical stage and move it back and forth. Adjust the position of the slide so that the specimen is over the condenser. Looking from the side, move the 10X objective lens up or down so that it positioned over the slide.

6. Adjust the light intensity, and looking through the ocular lens, move the stage up or down with the focusing knobs until the cells come into sharp focus. Use the course focusing knob first and then the fine focusing knob. **Be careful not to jam the objective onto the slide.** Adjust the light with the power switch and the iris diaphragm so you can see the cells clearly. **What is the total magnification of the image of the sample at this point?**

   _____

7. Slowly move the slide to the right with the mechanical stage. Which way do the cells move?

   _____

   Move the slide to the left. Which way do the cells move?

   _____

   Move the slide upwards. Which way do the cells move?

   _____

   Move the slide downwards. Which way do the cells move?

   _____

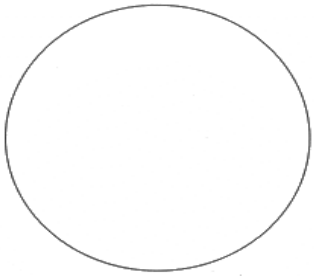
   What does this tell you about the formation of the image of an object in your microscope?

8. Rotate the nosepiece to bring one of the medium power objective lenses (20X or 40X) into position. These microscopes are designed to be parfocal, which means that the image should remain approximately in focus when you change objectives. However, you may find that you need to adjust the focus with the fine focusing knob to see the cells clearly. **What is the total magnification with this objective?**

   _____

   Make a drawing of some of the cells at this magnification.

Using the following circle to represent the microscopic field, draw the cells on the prepared slide of the stained animal tissue as seen with the 20X or 40X objective with bright field illumination. **Indicate the total magnification. Record your observations next to the circle.**



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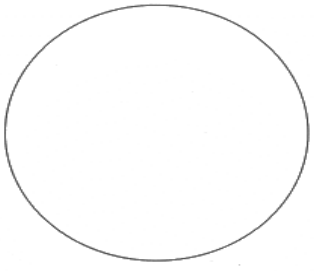
9. Turn the nosepiece so that the objective lens is away from the sample (sample should be between the 40X and 100X objectives). Do not move the stage at this point. In this way, the cells will remain centered under the nosepiece. Place a small drop of immersion oil directly on the slide. Rotate the nosepiece to bring the high power oil immersion objective (100X) into position. Looking from the side, use the fine focusing knob to move the objective close enough to the slide that the end of the objective just meets the oil. You will see the oil spread when this happens. Then, while looking through the ocular lenses, bring the image into focus with the fine focusing knob. The plane of focus with this objective is very thin so be careful. Note that you should only use oil with the 100X objective lens. What is the total magnification at this point?

_____

Make a drawing of some of the cells at this magnification.

Using the following circle to represent the microscopic fields, draw the cells on the prepared slide of the stained animal tissue as seen with the 100X objective with bright field illumination. **Indicate the total magnification. Record your observations next to the circle.**



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10. When you have finished looking at the slide, carefully wipe the oil off with a Kim-Wipe. Return the slide to the stock tray on the side bench. Clean the 100X objective with the special cleaning solution and lens tissue.

B. Calibration of the Ocular Micrometer

The objective of this part of the lab is to calibrate a scale (an ocular micrometer or reticle) in the right eyepiece of the microscope so that it can be used to measure the dimensions of different types of cells. Only some of the microscopes in the laboratory have this reticle, so be sure to use one of those microscopes for this part of the experiment.

1. As noted above, the stage micrometers we will be using have a scale that is divided into many large 1 mm units. Some of these 1 mm units are then divided into smaller 0.1 mm units. Some of the smaller 0.1 mm units are then divided into 0.02 mm units. **An example of one of these stage micrometers will be available as a demonstration slide under a dissecting microscope. Look at the scale closely so that you can see how it is organized.**

2. Rotate the 10X objective lens of one of the microscopes fitted with an ocular micrometer (reticle) into position. Obtain a stage micrometer and position it on the stage. **Be sure that the scale is on the top side of the slide.** Adjust the illumination and bring the image of the stage micrometer into focus. Rotate the ocular lens so that scale is straight. Note that the ocular micrometer has a total 100 units, divided into 10 unit and 1 unit intervals, so that the total is 100.

3. Move the stage to align the two scales near one another. Now define the relationship between the stage micrometer scale and the ocular micrometer scale. That is, how many units in the ocular scale are equal to a certain number of mm or μm in the stage micrometer?

4. What is your calibration value for the 10X objective?

   _____

5. Now rotate the 20X objective into position and repeat the process. What is your calibration value for the 20X objective?

   _____

6. What is the ratio of the two calibration values? Should this be a surprise?

   _____

7. Now rotate the 40X objective into position and repeat the process. What is your calibration value for the 40X objective?

   _____

8. What is the ratio of the two calibration values? Should this be a surprise?

   _____

9. **Do <u>not</u> attempt to calibrate the 100X oil immersion objective.** The stage micrometer is very expensive and it can be easily damaged. Based on your first two attempts at calibration, define a calibration value for this objective based on the change in magnification.

_____

10. Fill in the following **summary chart** with the calibration factors for your microscope as calculated above.

Objective Lens	Total Magnification	µm/Ocular Unit
10X		
20X		
40X		
100X		

C. Microscopic Observations of Human Cheek Cells

The objective of this part of the lab is to examine human buccal (cheek) epithelial cells using different optical techniques. Because cheek cells are transparent, it will be necessary to stain them in order to see them clearly by bright-field light microscopy or to use some of the other optical methods that enhance the contrast between the cells and the surrounding solution.

1. Obtain a clean glass slide and place a small drop of water (about 15-20 µl) in the center of the slide. Using the broad end of a flat toothpick, carefully scrap the inside of your cheek and then suspend the cells in the drop of water. Place a coverslip on top of this "wet mount" preparation, as shown in figure 2.10.

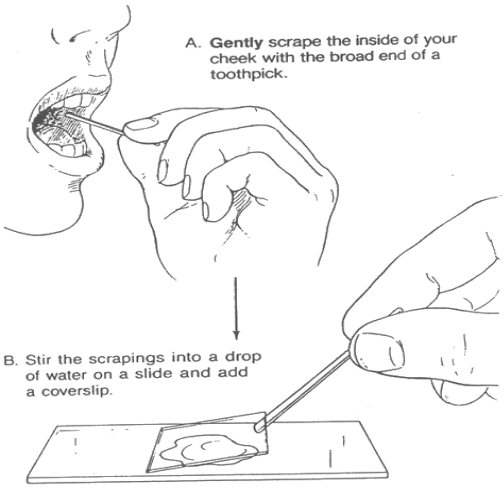
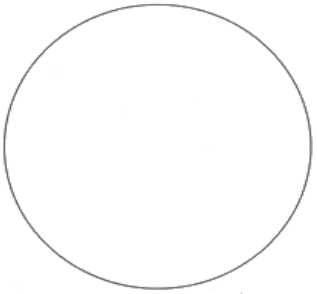


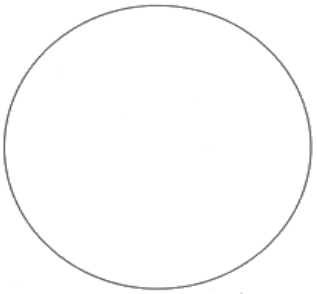
Figure 2.10. Preparation of human epidermal cells for microscopic examination.

2. Place the slide on the stage and move the specimen into position. Examine the cells first with the low power objective lens (10X). Because the cells are transparent, you will find it helpful to reduce the light intensity in order to see the cells clearly. Then switch to one of the medium power objective lenses (20X or 40X) and adjust the light intensity again**. Indicate the total magnification. Make a drawing of what you see, also write down observations you may have**.



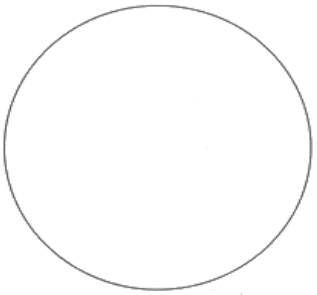
Bright Field _____X

3. With the slide still in the same position, move the turret on the condenser to the dark-field (DF) position. What do you see now when you look at the specimen? You may need to increase the illumination to see the cells clearly. You can either increase the power to the light source or open up the iris diaphragm. **Indicate the total magnification. Make a drawing of what you see, also write down observations you may have**.



Dark Field _____X

4. With the slide still in the same position, move the turret on the condenser to the PH1 (for the 20X objective) or PH2 (for the 40X objective) position. This introduces the correct phase plate for each objective. What do you see now when you look at the specimen? You may need to adjust the illumination to see the cells clearly. **Indicate the total magnification. Make a drawing of what you see, also write down observations you may have**.



Phase Contrast _____X

5. Now rotate the turret on the condenser back to the bright-field (BF) position and reduce the illumination. Remove the slide from the stage. To stain the cells, place a small drop of methylene blue stain on the side of the coverslip. Use a piece of paper toweling to draw the stain under the coverslip (Figure 2.11).

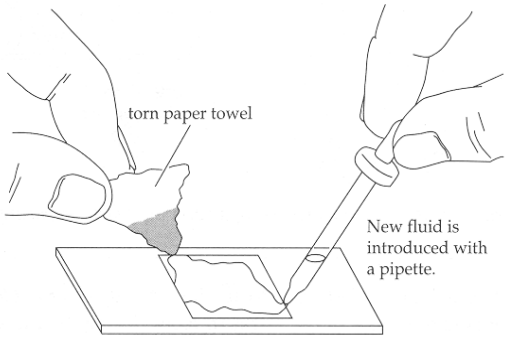
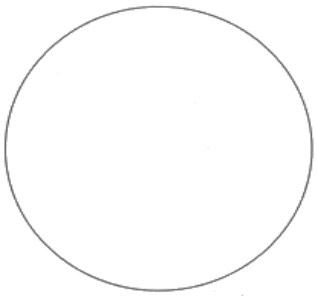


Figure 2.11. The solution under a coverslip can be changed without removing the coverslip by using a piece of tor paper towel to absorb the fluid at one corner edge of the coverslip, and introducing new solution with a pipette or eyedropper near the opposite corner.

6. Place the slide back on the stage and move the specimen into position. Examine the cells first with the low power objective lens (10X). Adjust the light intensity in order to see the cells clearly. Then switch to one of the medium power objective lenses (20 X or 40X). **Indicate the total magnification. Make a drawing of what you see, also write down observations you may have**.



Stained Cells _____X

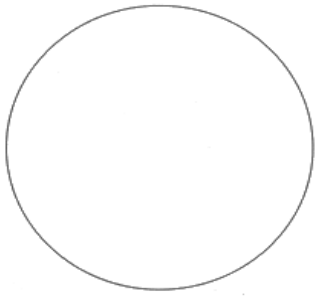
7. With the **40X objective lens** in the BF position, measure the diameters of 10 cheek cells using the calibrated ocular micrometer or reticle. Record the data below. The cells are a little irregular but they can be treated as if they were approximately circular. Avoid cells that are curled over.

Cell	Diameter (ocular micrometer units @____X)	Cell	Diameter (ocular micrometer units @____X)
1		6	
2		7	
3		8	
4		9	
5		10	

8. After you have measured the diameter of 10 cells, calculate the average or mean, the variance, and the standard deviation. **Refer back to Laboratory 1 (Scientific Calculations and Basic Techniques) for the information on these calculations if necessary.** Then, using the calibration or conversion factor you defined in Section IVB, convert the arbitrary ocular micrometer units into an actual diameter in µm.

	Ocular Units	µm
Mean		
Variance		
Standard Deviation		

9. Leaving the slide in position, move the 20X or 40X objective out of the way and add a small drop of immersion oil to the cover slip. Rotate the nosepiece to bring the high power 100X oil immersion objective into position. Looking from the side, use the coarse adjustment to move the objective close enough to the slide that the end of the objective just meets the oil. You will see the oil spread when this happens. Then, looking through the ocular lenses, bring the image into focus with the fine focusing knob. The plane of focus is very thin with this objective so be careful. **Indicate the total magnification. Make a drawing of what you see at this magnification.**



Stained Cells _____X

10. Now measure the diameters of 10 nuclei in the cheek cells with the ocular micrometer. Record the data below.

Magnification Used _____X

Nucleus	Diameter (ocular micrometer units)	Nucleus	Diameter (ocular micrometer units)
1		**6**	
2		**7**	
3		**8**	
4		**9**	
5		**10**	

11. Finally, calculate the mean for the diameter of the nuclei, the variance, and the standard deviation. Then convert the values to an actual diameter in µm using the correct calibration or conversion factor for the 100X objective.

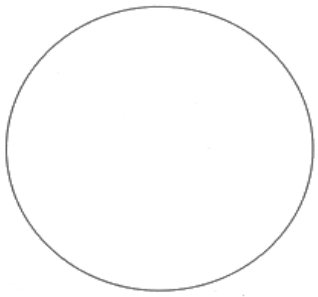
	Ocular Units	µm
Mean		
Variance		
Standard Deviation		

D. Microscopic Observations of *Elodea* Cells

The objective of this part of the lab is to examine photosynthetic cells in a leaf from the aquatic plant *Elodea*. Because these cells are intrinsically green, it will not be necessary to stain them in order to see them clearly by bright-field light microscopy.

1. Obtain a clean glass slide and place small drop of water in the center of the slide. Place a single leaf of *Elodea* in the water and carefully place a coverslip on top of this wet mount.

2. Place the slide on the stage and move the specimen into position. Examine the cells first with the low power objective lens (10X). Adjust the light intensity in order to see the cells clearly. Then switch to one of the medium power objective lenses (20X or 40X). Using the following circle to represent the microscopic field, draw an example of the Elodea cells in the wet mount as seen with the 20X or 40X objective. **Indicate the total magnification. Make a drawing of what you see, also write down observations you may have**.



Bright Field _____X

3. Look closely at the cells for evidence of cyclosis, which is the movement of the cytoplasm around the edge of the cell. This is usually visible in fresh leaves and can be detected by the movement of the chloroplasts. The chloroplasts appear to go around the edge of the cell, just underneath the cell wall, because much of the cytoplasm is occupied by a large central vacuole. **Does movement occur in all of the cells? Does it occur in the same direction in the cells in which it occurs? Record your observations.** If not observed ask instructor regarding this.

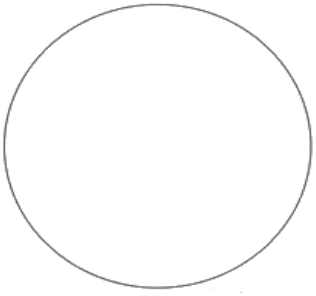
4. With the slide still in the same position, measure the length and width of 10 cells with the ocular micrometer. Record your data using a table with the following headings:

Cell	Length (ocular micrometer units @ ____X)	Width (ocular micrometer units @ ____ X)
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

5. As before, calculate the mean length and mean width for the 10 cells, the variances, and the standard deviations. Then convert the arbitrary ocular micrometer units (omu) to actual values in μm using the appropriate calibration or conversion factor.

	Length		Width	
	Ocular Units	**μm**	**Ocular Units**	**μm**
Mean				
Variance				
Standard Deviation				

6. Leaving the slide in position, move the 20X or 40X objective out of the way and add a drop of immersion oil. Rotate the nosepiece to bring the high power oil immersion objective (100X) into position. Looking from the side, use the coarse adjustment to move the objective close enough to the slide that the end of the objective just meets the oil. You will see the oil spread when this happens. Then, looking through the ocular lenses, bring the image into focus with the fine focusing knob. Using the following circle to represent the microscopic field, draw an example of the Elodea cells in the wet mount as seen with the 100X objective. Indicate the total magnification and write down observations you may have.



Bright Field _____X

7. Using the oil immersion objective, measure the diameter of 10 chloroplasts with the ocular micrometer. Make up a table similar to that you have used before.

Chloroplast	Diameter (ocular micrometer units @ _____X)
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	

8. Finally, calculate the mean for the diameter of the chloroplasts, the variance, and the standard deviation. Then convert the values to an actual diameter in µm using the correct calibration or conversion factor for the 100X objective.

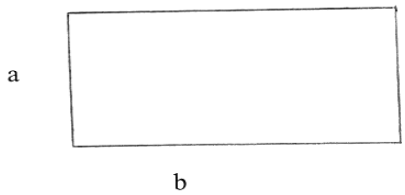
	Ocular Units	µm
Mean		
Variance		
Standard Deviation		

E. Calculation of Cell Volumes

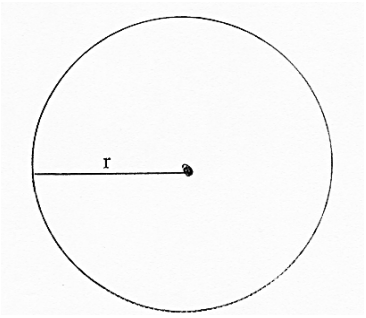
While macromolecules, cellular organelles, and whole cells have complex three-dimensional structures, their shapes can be approximated by simple geometric forms. It is often both useful and necessary to make calculations based on these forms. The objective of this part of the lab is to do some simple calculations based on the measurements that you have made on the human cheek cells and *Elodea* cell.

1. Area and Volume of Two Dimensional Objects

a. For a **square** or **rectangle**, the area is the product of its dimensions on each side, so A = (a) (b):



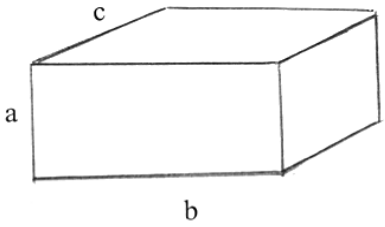
b. For a **circle**, the area (A) is πr^2, where $\pi = 3.14$ and r is the radius of the circle or 1/2 its diameter.



2. Area and Volume of Three-Dimensional Objects

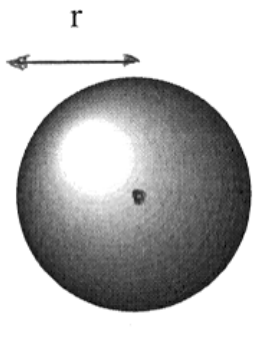
a. For a **cube** or **rectangular solid**, the total surface area is the sum of all of the areas of the individual sides.

$A = 2\,(a)(b) + 2\,(a)(c) + 2\,(b)(c)$



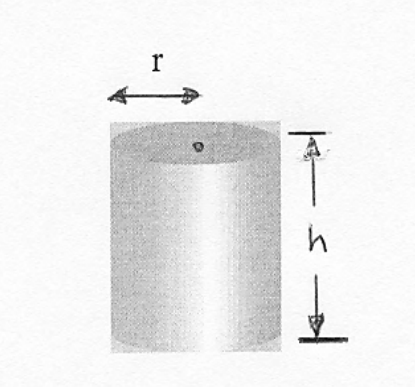
The volume is the product of the three linear dimensions, so V = (a) (b) (c).

b. For a **sphere**, the surface area is $4\,\pi r^2$, where r is the radius of the sphere or 1/2 its diameter.



The volume of a sphere is $4/3\,\pi r^3$, where r is again the radius of the sphere or 1/2 its diameter.

c. For a **cylinder**, the volume is $\pi r^2 h$, where r is the radius of the circular end and h is the height of the cylinder



3. Calculation of areas and volumes. A bacterium called *Staphylococcus aureus* is a spherical cell about 2 μm in diameter.

a. What is its area in m^2?

b. What is its area in nm^2?

c. What is it area in mm^2?

d. What is its volume in m^3?

e. What its volume in nm^3?

f. What is its volume in mm^3?

Solutions to the above problems:

a. Since the diameter is 2 m, the radius is 1 m.

$$A = 4 \pi r^2 \quad = \quad 4 (3.14) (1\ \mu m)^2 \quad = \quad 12.56\ \mu m^2$$

b. Since each m contains 1000 nm, 1 μm^2 = (1000 nm)(1000 nm)
= $(10^3$ nm) $(10^3$ nm)
= $10^6\ nm^2$

$$12.56\ \mu m^2 \quad x \quad \frac{10^6\ nm^2}{\mu m^2} \quad = \quad 12.56 \times 10^6\ nm^2$$

$$= \quad 1.26 \times 10^7\ nm^2$$

c. 1 μm = 0.001 mm, 1 $\mu m^2 = (10^{-3}$ mm$)(10^{-3}$ mm) = $10^{-6}\ mm^2$

$$12.56\ m^2 \quad x \quad \frac{10^{-6}\ mm^2}{\mu m^2} \quad = \quad 12.56 \times 10^{-6}\ mm^2$$

$$= \quad 1.26 \times 10^{-5}\ mm^2$$

d. Since the diameter is 2 μm, the radius is 1 μm.

$V = 4/3\ \pi r^3 = 4/3\ (3.14)\ (1\ \mu m)^3$
$= 4.19\ \mu m^3$

e. Since each m contains 1000 nm, $1\ \mu m^3 = (1000\ nm)(1000\ nm)(1000\ nm)$
$= (10^3\ nm)\ (10^3\ nm)\ (10^3\ nm)$
$= 10^9\ nm^3$

$$4.19\ \mu m^3 \times \frac{10^9\ nm^3}{\mu m^3} = 4.19 \times 10^9\ nm^3$$

f. Since 1 μm = 0.001 mm, $1\ \mu m^3 = (10^{-3}\ mm)(10^{-3}\ mm)(10^{-3}\ mm)$
$= 10^{-9}\ mm^3$

$$4.19\ \mu m^3 \times \frac{10^{-9}\ mm^3}{\mu m^3} = 4.19 \times 10^{-9}\ mm^3$$

Do the following additional calculations based on today's observations in the lab. **Record the answers in your lab notebook.**

1. Based on your previous calculation of the mean diameter of 10 human cheek cells, determine the volume of an average human cheek cell in μm^3 assuming that it is a sphere.

2. Human cheek cells are actually flattened and stacked on top of one another in what is called simple squamous epithelium. Assuming that the thickness of an average human cheek cell is 5 m, what is the mean volume of an average human cheek cell in μm^3? Use the formula for a cylinder.

3. Based on your previous calculation of the mean diameter of 10 human cheek cell nuclei, calculate the average volume of a human cheek cell nucleus in μm^3. Assume it is a sphere.

4. If a flattened human cheek cell (#2) were filled only with nuclei, how many could it hold?

5. Based on your previous measurements of the mean length and width of 10 *Elodea* cells, determine the average volume of these cells in μm^3 assuming they are rectangular solids. Assume that the thickness of the cell is the same as its width.

6. How does the volume of an Elodea cell compare to the volume of a human cheek cell? What is their ratio?

7. Based on your previous calculation of the mean diameter of 10 *Elodea* chloroplasts, calculate the average volume of a chloroplast in μm^3 assuming it is a sphere.

8. How does the size of an *Elodea* chloroplast compare to the size of a cheek cell nucleus? What is the ratio of the two volumes?

9. If an *Elodea* cell were filled only with chloroplasts, how many could it hold?

10. If an *Elodea* cell were filled only with nuclei the same size as a cheek cell nucleus, how many could it hold?