

## Lab Exercise 1: The Microscope & Scientific Measurements

### Background

A **microscope** is used to look at things that are too small to be **resolved**, or seen, with the naked eye. As such, the microscope is one of the most important tools to microbiologists. In this laboratory, we utilize a **compound optical microscope**. Compound, because it has more than one lens and optical because light is what transmits the image to your eye. Although it is difficult to pinpoint the exact origin of the microscope, Robert Hooke and Anton van Leeuwenhoek are both credited with bringing the microscope to the field of biology.

Today's microscopes are a far cry from those invented in the 17<sup>th</sup> century and a typical student compound optical microscope will have the capability of magnifying an object up to 1000 times (1000×) its size. Even with all of the modern advancements, the principles of microscopy remain the same. The total magnification of a given object is the product of the ocular lens power and the objective lens power. The Nikon Alphaphot-2, used in this class, has objectives capable of magnifying a specimen 4×, 10×, 40× and 100× its size. In addition, your oculars contain a lens that increases the magnification of the object you are viewing by 10×. Thus, your actual magnification is the product of these two values (Table 1).

**Table 1: The corrected magnification levels for use when viewing an object with the Nikon microscope. Each of these represents the product of the individual objective lens and the 10× ocular.**

Lens	Actual Magnification
4×	40×
10×	100×
40×	400×
100×	1000×

**Resolving power** is also an important consideration. **Resolution** is the ability to distinguish two closely spaced objects as distinct and separate. When magnification exceeds resolving power, the object in view is blurry. The resolution of the human eye is approximately 300µm but the resolving power of your microscope is closer to 0.2µm when the 100× objective is used.

Two more things to consider when viewing an object with your microscope are **field of view (FOV)** and **depth of view (DOV)**, both of which have an inverse relationship to magnification. Field of view refers to the width and height of the object that can be seen under the microscope. Field of view decreases proportionately as magnification increases. For instance, your FOV at 100× (using the 10× objective and looking through 10× oculars) is 2.5 times smaller than the FOV of the 4× objective lens. Additionally, depth of view- which refers to the thickness of the plane in focus- changes similarly with a change in magnification. As the magnification is increased, the DOV decreases and you are able to see a thinner slice of the microscope slide-specimen under view. This is one reason why even a **parfocal microscope** (one whose lenses stay in focus when the magnification is changed) must be fine focused when the objective is changed. It is also the reason that a slide placed on the microscope stage upside down will be visible under low magnification but impossible to see using higher magnification. Keep this in mind if you ever find yourself in a position where specimens “disappear” when you try to find them using anything other than your 10× objective.

As mentioned above, as you increase the magnification of the specimen you are viewing, you also reduce your field of view- by a corresponding but equal factor. Likewise, if you decrease the magnification (by using an objective with a less powerful lens) you increase the field of view. Sometimes it is easier to understand this relationship by thinking of something a bit larger than the area seen under a microscope. If you imagine looking at a hillside with the naked eye and then with a pair of binoculars, you will agree that the binoculars allow you to see a smaller portion of the hillside in much greater detail. The microscope works the same way. Using the 10× objective, you will see a smaller portion of the field of view in greater detail than that provided by the 4× objective. In other words, the 10× objective magnifies objects 2.5× larger than the 4× objective and it reduces the field of view by 2.5×. This relationship is easily computed, as 10 is 2.5 multiplied by 4. These relationships are important to understand before you begin today's laboratory activity because you will only be able to measure the field of view for a single objective and you will have to mathematically extrapolate the field of view for the remaining three objective lenses.

Learning to estimate the size of objects being viewed under the microscope is an important tool in the microbiological laboratory. Although the process is slightly more complicated than measuring the distance from one end of the laboratory to the other, the basic principle is the same. Determine the proper tool to measure the length you wish to measure and then use that ruler to measure the distance. One of the benefits of using the **International System of Units (SI)** is that it is a **decimalized system of measurement**. Each of the three measurements: volume, distance and mass have a basic unit of measurement. Each also uses a standard set of prefixes which relate to the basic unit by some factor of 10 (Table 2). Those students who are familiar with Latin and Greek may be interested to know that all Latin prefixes indicate a value by which the basic unit is divided (e.g., centi-) and all Greek prefixes indicate a value by which the basic unit should be multiplied (e.g., kilo-).

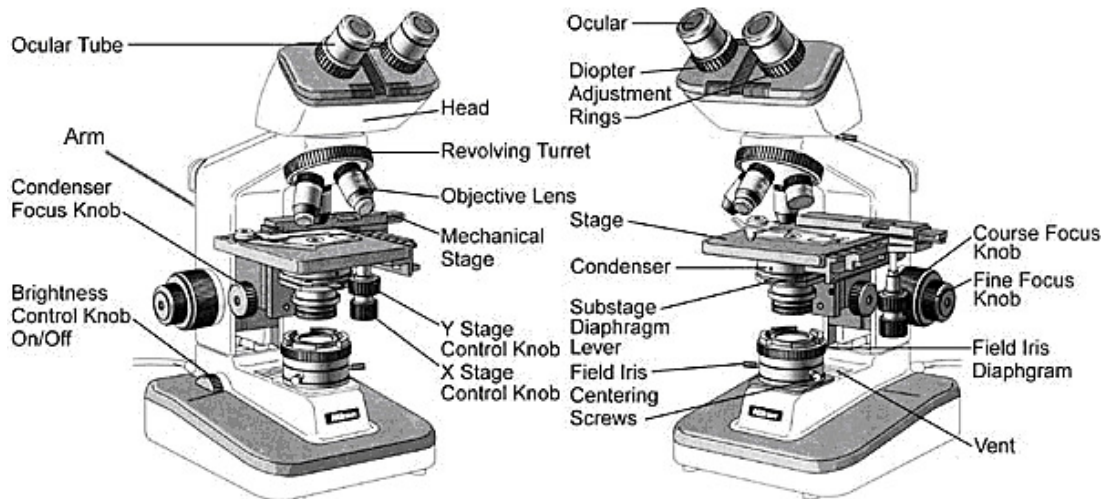
**Table 2: Table indicating some of the basic prefixes used in the SI (metric) system of measurement.**

Prefix	Abbreviation	Scientific Notation	Fractional Equivalent	Decimal Equivalent
kilo-	k	$1 \times 10^3$	1000	1000
centi-	c	$1 \times 10^{-2}$	1/100	0.01
milli-	m	$1 \times 10^{-3}$	1/1000	0.001
micro-	$\mu$	$1 \times 10^{-6}$	1/1000000	0.000001
nano-	n	$1 \times 10^{-9}$	1/1000000000	0.000000001
pico-	p	$1 \times 10^{-12}$	1/1000000000000	0.000000000001

When measuring the length of the field of view of your microscope, the basic unit of measurement will be the **meter**. Because your microscope measures things that are quite a bit smaller than a basic meter, you will use the prefixes associated with a fraction of the meter, namely the **millimeter** (mm) and the **micrometer** or **micron** ( $\mu\text{m}$ ).

## Introduction

You will be assigned a Nikon Alphaphot-2 Compound Light Microscope (Figure 1) for your use during the semester. Although you will share this microscope with one person from every other laboratory section, you will not share this microscope with any of the students in your class. This microscope is equipped with a 10 $\times$  ocular and four objective lenses (4 $\times$ , 10 $\times$ , 40 $\times$  and 100 $\times$ ) which allow you to view objects at 40 $\times$ , 100 $\times$ , 400 $\times$  and 1000 $\times$  their actual size. Because the organisms we study in lab are typically prokaryotic bacteria only a few micrometers in size, you will most often use the highest magnification that your scope is capable of in order to see them.



**Figure 1: Labeled diagram of the Nikon Alphaphot-2 student microscope.**

## Parts of the compound microscope

### Light source

A light microscope uses visible light to view the specimen. Light intensity is controlled by both a **Power Switch** and a **Brightness Control Knob**. It is general practice to turn on the power and then turn the brightness to maximum. The light will be regulated into the scope using an **iris diaphragm** by adjusting the **substage diaphragm lever**.

### Condenser

The **condenser** is composed of 2 sets of lenses found directly below the stage, which collect and concentrate the light upward into the lens systems. The iris diaphragm is located within the condenser and will further regulate the amount of light directed through the lens system. On the side of the substage diaphragm lever you will find four number designations which correspond to the four **objective lenses**. The lever should always be adjusted to match the objective (*i.e.*, if you are using the 4 $\times$  objective, turn the lever to the number 4). The magnification is inversely related to the amount of light allowed through the objective lens, so as magnification increases, the lever diaphragm must open to allow increased light into the lens system, compensating for the smaller hole in the objective. In addition to the diaphragm adjustments, adjustments can be made to the condenser directly using the **condenser focus knob**- it is suggested however that you keep the condenser in its fully raised position.

### Objective Lenses

This is the first set of lenses that will magnify the specimen for viewing. They are housed beneath the **revolving turret**, which can be mechanically turned to allow you to change the objective lens. The Nikon Alphaphot-2 is a **parfocal** microscope. This allows you to change the objective lens without having to make major lens focusing adjustments. By

definition, a parfocal microscope should only have to be brought into focus using both the **coarse focus knob** and **fine focus knob** with the initial objective lens. Increasing the magnification by switching to a higher magnification objective should require only  $\frac{1}{2}$  forward turn of the fine focus knob. Unfortunately, as the microscopes get used, they tend to lose a bit of their fine adjustment and may require up to 10 turns in either direction when the objective lenses are changed.

Each objective has a small opening through which light from the microscope is collected to view the specimen on your slide. For the 4–40 $\times$  objectives, this hole is large enough to permit adequate light to pass through. These objectives are called **dry objectives**, with the 40 $\times$  objective referred to as “**high dry**.” The hole in the 100 $\times$  objective is too small to allow adequate light into your microscope, so oil is used to refract additional light into the scope to allow viewing. This lens is therefore referred to as an **oil-immersion lens**. You should never use this lens without oil and the oil should always create a physical continuum between the slide and your objective. Additionally, you should never use oil with any of the dry objectives.

### Stage

This is a platform on which the microscope slide rests and is centered over the light source. The mechanical portion of the stage can be adjusted either vertically or horizontally to center the specimen over the light source, using the **Y stage (vertical)** and **X stage (horizontal) control knobs**. The stage is connected to the microscope body and is raised and lowered to bring objects into fine focus using first the coarse and then the fine focus knobs.

### Head

The head of the microscope rotates, but should always be kept facing forward. The head houses the **eyepieces (ocular lenses)** and the **diopter adjustment rings**. When using your microscope, it is important to note that the eyepieces adjust horizontally to compensate for your interpupillary distance (*i.e.*, the distance between your eyes). In addition, the diopter adjustment rings will allow you to compensate for differences in vision between your eyes.

### Arm

Microscopes are stored in the cabinet with the **arm** facing outwards. Always lift the scope by this arm and place a second hand under the base to transport it. When removing your scope from the cabinet, **DO NOT LET THE OCULARS HIT ANY PART OF THE CABINET**. Carefully transport the microscope to your station and gently place the microscope on your bench with the oculars toward you. **NEVER PUSH OR DRAG THE MICROSCOPE ON THE BENCHTOP, ALWAYS LIFT IT TO MOVE IT**. Pushing or dragging the microscope on the bench top causes it to BOUNCE which jars and damages the optical systems of the microscope.

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## Introduction

For today’s laboratory activity, you will be using a microscopic ruler called a **stage micrometer** in order to measure the field of view of your microscope. The stage micrometer is a microscope slide containing a ruler whose basic unit of measurement is the micrometer (Figure 2).

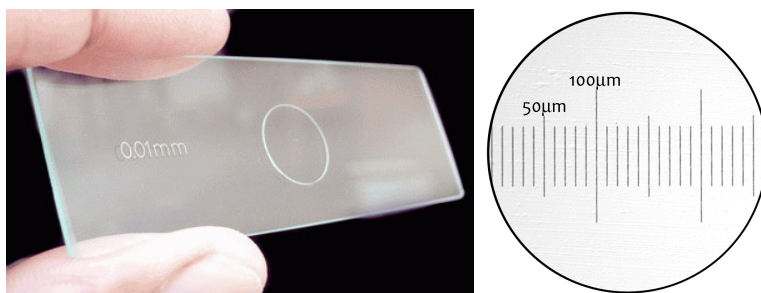


Figure 2: Picture (left) and illustration (right) of the microscopic view of the stage micrometer to be used in today’s lab. Note that the units depicted in the right-hand illustration are micrometers with each tick mark representing 10 $\mu\text{m}$  (the label on these stage micrometers is 0.01mm, the equivalent of 10 $\mu\text{m}$ ). Light micrograph 800 $\times$ .

You will need to bring the ruler on the stage micrometer into view using a low-dry objective and then, using the **parfocal** qualities of your microscope, find it at the highest magnification at which you can accurately measure the length of your field of view. From there, you will extrapolate the distance for the remaining three objective lenses. Once you have successfully calibrated your microscope, you will determine the size of various microscopic species.

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## Objectives

1. Identify the parts of the compound optical microscope and their functions.
  2. Use a stage micrometer to measure the field of view (FOV) of your microscope.
  3. Use the microscope to locate and estimate the size of various organisms on prepared slides using the FOV data collected during the *Field of View Calibration Protocol*.
  4. Properly clean and store the microscope.
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## Pre-Lab Calculations

- Using the information in the *Background* section, relate the magnification of each of the lenses to each other in the table below.

objective lens	related to 4×	related to 10×	related to 40×	related to 100×
4×	1			
10×	2.5			
40×	10			
100×	25			

- Using the relationship between fields of view determined above, record the FOV measurements for each objective lens. The data below are an EXAMPLE.

ocular magnification	objective magnification	total magnification	FOV (μm)
	4×		
	10×		200
	40×		
	100×		

## Protocol

Individual Supplies
stage micrometer
at least 3 prepared eukaryotic microbe slides

## Microscope Use Protocol

### Viewing Your Specimen

- Plug in your microscope, taking care to insure the cord is firmly attached to the back of the scope.
- Turn on the power switch. Increase the light intensity dial until it is at its maximum position.
- Place a microscope slide on the stage and secure it in place against the corner of the stage using the spring-loaded arm (the slide should sit against this arm, not under it). Center the slide over the light source using the X and Y stage adjustment knobs.
- Rotate the revolving turret until the 4× objective lens is locked in place over the slide. Make sure that the substage diaphragm lever is set to 4. Raise the stage to its top-most position.
- While looking through the eyepieces, adjust for your interpupillary distance by distancing the eyepieces until you can see one image.
- While still looking through the ocular lenses, slowly lower the stage using the course adjustment knob until the specimen comes into focus. If you are unsure whether the object you are viewing is on your slide, very slightly move the stage back and forth.
- Once you have the specimen in coarse focus, use the fine focus knob to bring the specimen into sharp focus. Using the X and Y stage adjustment knobs, center the object in your field of view. *Note: If you have markedly different eyesight between your eyes, close one eye and adjust the fine focus until the object is in sharp focus. Close the other eye and adjust the focus knob on the end of your eyepiece until the object is again in sharp focus.*
- Rotate the revolving turret to the 10× objective. Adjust the substage diaphragm lever to 10. Bring the specimen into the center of your field of view. *Note: Because of the parfocality of the microscope you should only have to adjust the fine focus knob ½ turn forward to bring the specimen into sharp focus. If this does not work, slowly rotate the fine focus knob first forward about 10 turns and then backward to zero and backward 10 turns. Make note of the adjustment you made, this should remain constant throughout the semester as long as you are using this microscope.*
- Rotate the revolving turret to center the 40× objective. Adjust the substage diaphragm lever to 40. Bring the specimen into the center of your field of view. Once your specimen is in focus at 40×, you are ready to observe the sample using the 100× lens. *Note: Because the 40× objective has a small opening, it is often difficult for adequate light to enter the objective. This results in blurry and dark images that oftentimes cannot be corrected. This problem should be resolved once you view the*

specimen using the 100× objective because the added oil will cause increased light to refract up into the objective lens, providing a clearer, sharper image of your specimen.

10. Prepare to view the specimen using the 100× objective by rotating the revolving turret so that the space between the 40× and 100× objective is directly over your slide.
11. Add one drop of immersion oil directly to the slide.
12. Slowly move the 100× lens into place. **THE 100× LENS SHOULD BE FULLY IMMERSSED IN THE OIL.**
13. Using the fine focus knob, bring the object into sharp focus. *Note: Once you have added oil to the slide, you can no longer use your 40× objective. If you cannot find your specimen, you must rotate to 10× only. Do not allow the 40× to pass by the slide again or it will be contaminated with oil.*

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### Field Of View Calibration Protocol

1. Place the stage micrometer on the stage and center it in the light path.
2. Determine which of the four objectives (4×, 10×, 40× or 100×) will allow you to measure the field of view of your stage micrometer most accurately. Hint: This will be the lowest power objective where the entire field of view is within the micrometer marks.
3. Bring the stage micrometer into sharp focus using this objective. Using the X and Y stage control knobs, make sure that the center of the stage micrometer is at the diameter of the field of view. Make a note in your lab notebook of which objective you are using.
4. Determine the Field of View (FOV) for this objective by counting the number of delineations visible across the field of view. Each of the delineations is equal to 10µm or 0.01mm apart, this means that the number of tick marks must be multiplied by 10 to get the correct number of µm.
5. To calibrate your scope accurately for the remaining three fields of view, you must extrapolate from the FOV measurement collected above. In order to do this, it is important to remember that the change in magnification is inversely proportional to the change in FOV (e.g., If the magnification increases by a factor of 10, the FOV decreases by a factor of 10).
6. Complete the table below before continuing to the *Estimating the Size of Eukaryotic Microbes Protocol*.

ocular magnification	objective magnification	total magnification	FOV (µm)
	4×		
	10×		
	40×		
	100×		

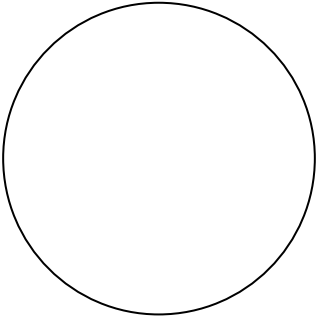
### Estimating the Size of Eukaryotic Microbes Protocol

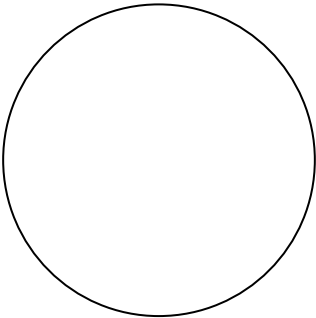
1. Using the directions outlined in the *Viewing Your Specimen Protocol* above, locate and bring into focus at least 3 of the prepared eukaryotic microbe slides.
2. Using the field of view calibrations, estimate their size.
3. Place these drawings in your lab notebook, making sure to note total magnification.

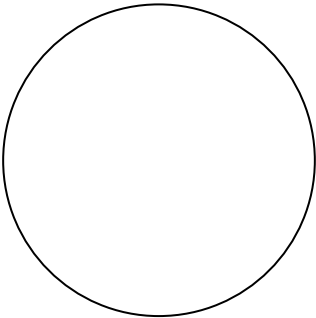
### Putting Away Your Microscope

1. Using the brightness control knob, turn down the light completely.
2. Turn off the microscope.
3. Unplug and wrap the cord around the microscope.
4. Using the coarse focus knob, lower the stage completely.
5. Using LENS PAPER and LENS CLEANER clean the objective lenses. There should only be oil on the 100× objective, but confirm that there is no oil on any of the objectives or the stage.
6. Turn the revolving turret so that the 4× objective is directly above the stage. Using the X and Y stage adjustment knobs, move the stage in a position close to the arm.
7. Carefully place your microscope in its designated cupboard space with the oculars facing into the cupboard.

## Data Collection and Analysis

Organism name	Estimated size in $\mu\text{m}$	Drawing
		 <p>Total magnification _____ <math>\times</math></p>

Organism name	Estimated size in $\mu\text{m}$	Drawing
		 <p>Total magnification _____ <math>\times</math></p>

Organism name	Estimated size in $\mu\text{m}$	Drawing
		 <p>Total magnification _____ <math>\times</math></p>

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### Discussion

1. For what purpose would you adjust each of the following microscope components during a microscope exercise: iris diaphragm, coarse adjustment knob, fine adjustment knob, and mechanical stage control?
2. Why is it advisable to start first with the low power lens when viewing a slide?
3. Why is it necessary to use oil in conjunction with the 100 $\times$  lens but not the other objectives?
4. How do light, DOV and FOV change when you increase magnification?
5. What is the distance between each of the graduations of your stage micrometer?
6. Why does the actual magnification differ from the magnification listed on the objective lens?
7. Why must each microscope be calibrated individually? Will you ever have to calibrate your microscope again?