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MICROSCOPE USE AND YEAST CELLS

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I. OBJECTIVES:

1. To become familiar with the parts of a microscope.
2. To learn how to use a microscope.
3. To use the microscope to view yeast cells.

II. BACKGROUND:

Back in the 1600s, a number of scientists were working on the invention and improvement of the microscope. In 1663, Robert Hooke used this new tool to view a section of the bark of the cork oak tree (*Quercus suber*), and he saw many small compartments which he called "cells" (cell = a small room). Shortly thereafter, Antonie van Leeuwenhoek made use of the microscope (micro = small; scope = see, watch, look) to view many tiny organisms and cells for the first time. Today, the field of microscopy has improved considerably, and not only do we

have light microscopes like those we will be using in this lab, but also electron microscopes which use a beam of electrons rather than light to "see" a specimen.

Because microscopes are expensive and because of the number of other students in other lab sections/classes who also use these microscopes, it is important to follow a few simple rules that will insure that good care is taken of the microscopes. These rules and requirements will be covered in the procedure to follow.

III. MATERIALS NEEDED:

microscope – will be assigned based on where you are sitting. You should always use the same one, corresponding to your seat number.

prepared slide of the letter "e"
yeast culture
methylene blue
slides and coverslips

IV. PROCEDURE:

First, your instructor should

- show the class the microscope-use videos on the Biology Web site, and
- point out to you all the microscope parts listed here on an actual microscope and draw and label an illustration of the microscope on the board as you do the same in your lab notebook.



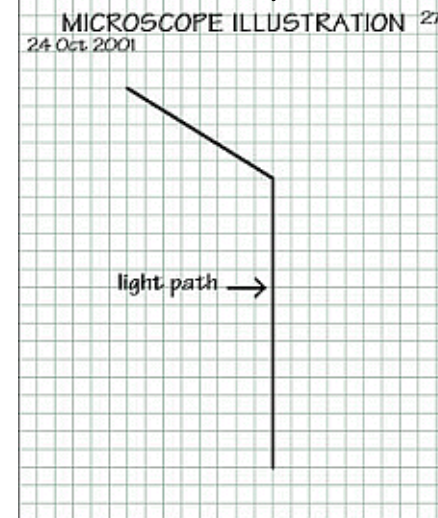
CARRYING AND STORAGE OF THE MICROSCOPE:

To lift/carry a microscope, grasp the arm firmly in one hand and lift/support the base with your other hand. Microscopes are located in the cupboard beneath your desk. When removing/replacing your microscope from/into the cabinet, be very careful not to bump it, especially the lenses, or you may damage it. Obtain the microscope that corresponds to your assigned seat number, set it on the desk top, and return the plastic dustcover to the cupboard. Because space in the cupboard is at a minimum, to avoid accidents, it is imperative that you properly coil the cord around the microscope. Hopefully, the last person stored it away that way, so observe the (hopefully) neatly-coiled cord on your microscope. Practice (yes, this may seem silly, but DO IT) wrapping and unwrapping the cord a couple times. The cord should be coiled in a clockwise direction. Make sure the cord is not twisted or kinked and that the end is securely tucked into the rest for storage. You are expected to make sure the cord is neatly coiled each time you put it away after use (this is a major source of rivalry

among the various lab sections) so that when your lab partner gets out his/her microscope, yours won't fall on the floor. If your microscope is not properly stored, rest assured that students in the other lab sections will complain to their instructor and blame you. When you are getting ready to use the

THE MICROSCOPE AND ITS PARTS:

Become familiar with your microscope and its parts. As your instructor goes through the names and locations of the microscope parts, in your lab notebook, draw a full-page-sized picture (right-side view) of the microscope you are using and label the following, underlined parts, noting their functions. As you go through the parts, fill out the appropriate places on the Microscope Check-in Sheet located in your drawer.



A. Note the path traveled by the light. Start your notebook illustration by indicating the light path as a (lower) vertical line connected to a (upper) line that angles to the upper left. This will help you to align the various parts as you draw them.

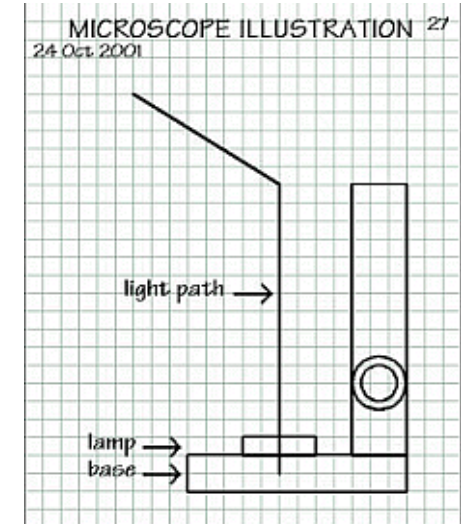
B. The base is the main support for the microscope.

C. The arm supports the stage and the optical head. Again, note that the proper way to carry a microscope is to grasp the arm with one hand and support the base with the other hand.

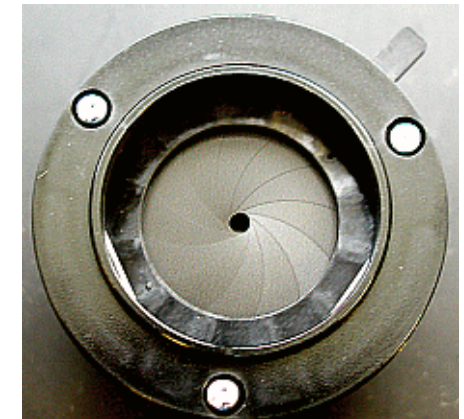
D. An electric light is mounted on the base of the microscope, and is controlled by both an on-off switch and a rheostat (rheo = flow, current) which adjusts the brightness. Please keep the light off when not in use to avoid heat build-up. If you finish looking at one slide and need to clean it off and/or go get another, turn off the light in between. Also,

microscope, as you are plugging in the cord, lay the excess across the tabletop – NOT dangling down in front of you and the cabinet door. If the cord is dangling down, the risk is great that you will accidentally trip over it, catch it as you attempt to open the door, etc., and pull the microscope off the table.

turn off the light before unplugging and storing the microscope. To extend the life of the bulb, when turning the light off, always lower the rheostat to the lowest light level before switching on or off the light.



E. The stage is the flat area upon which the specimen is placed. It has a hole in the center through which light may pass.



F. The iris diaphragm and condenser are located under the stage. The condenser focuses the light going through the specimen and the iris diaphragm is used to regulate the amount of light passing through the specimen

(which also, as in a camera, influenced the depth of field). Locate the iris diaphragm lever and the condenser height adjustment knob. Often, if you cannot see a specimen clearly, it is because the amount of light passing through needs to be greater or less.

G. Mounted on the stage is a mechanical stage which includes a specimen holder. This is designed to hold the slide in place so it doesn't move while you're looking at it and to move the slide around from place to place. Note the vernier scales that indicate the position of the slide (side-to-side and front-to-back) – jotting down these numbers makes it possible to return to an exact location on a slide. The mechanical stage is controlled by the low drive coaxial stage controls located on the lower right-hand side of the stage.

H. A pair of large knobs, called the coarse adjustment, which permit rapid raising or lowering of the stage, are located on the sides of the arm. Memorize the direction to rotate this knob to lower or raise the stage.

I. A pair of smaller knobs, called the fine adjustment, which permit smaller adjustments in the stage height are located “inside” the coarse adjustment knobs.

J. The optical head, the body of the microscope which contains the lenses, is bent at an angle on these microscopes. There is an optical head retaining screw which holds the optical head onto the microscope. **DO NOT LOOSEN THIS SCREW!** If it is left loose, the optical head will fall off (and land on the floor and break).

K. The nosepiece is a revolving plate on the lower side of the optical head which holds the objective lenses. Note that as it is rotated,

USE OF THE MICROSCOPE:

A. Place the microscope on the table in front of you with the arm away from you. Plug in the cord and drape the excess across the tabletop – NOT HANGING DOWN!!!

B. You may need to adjust the diaphragm (now or later) so that the proper amount of light passes through your specimen.

C. If the microscope was properly stored, the 4× objective should be in position in the light path (under the optical head). If not, swing the nosepiece around until it clicks into position.



Figure 5.
Letter “e”
specimen!

D. Obtain a prepared slide of the letter “e.” Observe (and draw) which way the “e” is mounted on the slide. Handle these slides only by the edges or the labeled end and use lens paper or a Kimwipe to clean if needed – don't put your thumbprint in the center of the specimen! Place the slide onto the stage,

each lens **CLICKS** into place. A frequent cause of students seeing “strange” images is an improperly seated objective lens.

L. On the nosepiece, a 4× scanning objective or lens (red band), a 10× low-power objective (yellow), a 40× high-power objective (blue), and a 100× oil immersion lens (white) may be found. **DO NOT USE THE OIL IMMERSION LENS UNTIL/UNLESS YOU HAVE RECEIVED SPECIFIC INSTRUCTIONS TO DO SO AND HOW TO USE IT.** Note that these lenses are color-coded, and remember which color is which for easy identification. **ONLY lens paper** (located in the drawer in front of you – let your instructor know if your supply is getting low so we can replenish it) should ever be used on the lenses, and only if absolutely necessary. **Never** use paper towel, Kleenex, or even Kimwipes on the lenses – ever!!! (NOTE: the drawer in front of you is NOT a trash receptacle – dispose of trash properly. Also, there are A&P slides and a bottle of immersion oil in the drawer – these are off limits to General and Introductory Biology students!)

M. The oculars (ocul = an eye) lenses fit into the top of the tube and are 10×. Be careful they don't fall out – they are not attached. The oculars can be moved closer together or farther apart to adjust to the width of your eyes. As you move them in and out, note the interpupillary distance scale which indicates how far apart they are spread. Note on the left, outer barrel of the left ocular there is a white line running up the side and some other markings, the diopter adjustment scale – you will use this to adjust the focus to your eyes.

specimen-side up. By looking from the side and the front, use the mechanical stage controls to try to place the “e” as close to the center of view (**optic center**) as you can.

E. Carefully, **LOOKING AT WHAT YOU'RE DOING FROM THE SIDE**, use the coarse adjustment to raise the stage as far as it will go without hitting the lenses – **DO NOT LET THE LENS TOUCH THE SLIDE. NEVER** use the coarse adjustment to raise the stage while looking through the microscope or you could smash the lens into the slide. Again, check from the side and front to align the “e” as closely as possible.

F. If you were successful at getting the “e” directly under the objective, you should see a faint haze when looking through the RIGHT ocular. Slowly, move the stage **DOWN** using the coarse adjustment (make sure you're going down, not up) until your specimen comes into view. If the “e” is not visible when you first look through the ocular, move the slide a little

one way or another while looking through the **RIGHT** ocular watching for a haze or dark spot to pass by. Back up to that spot, then proceed to focus.

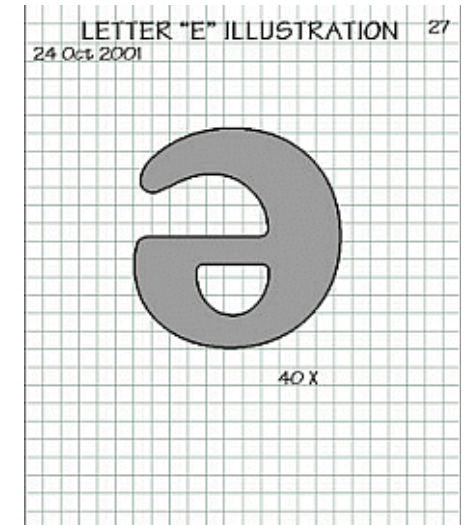


G. Make fine adjustments in the focus with the fine adjustment knob if needed so that what you see in the **RIGHT** ocular is in focus. On the base of the left (outer) side of the **LEFT** ocular, there are some shorter lines, a “0”, a “+”, and a “-”. Notice that, as you turn the diopter adjustment ring on the left ocular, various of the shorter lines line up with the longer line. With the “0” line matched up with the longer line, look through the **LEFT** ocular and turn it until it, too, is in focus (do NOT adjust the main focus knobs – you are adjusting now for differences between your two eyes). Hopefully, each eye should be in focus now. Next, you need to adjust the spread of the oculars to match your interpupillary distance by moving the oculars in or out until you can comfortably see the image through both eyes at the same time. In your lab notebook, draw the scales for both the diopter adjustment ring and the interpupillary distance scale, indicating the exact numbers/position that are correct for **YOUR** eyes. The next time you use your microscope, you can, then, adjust the microscope to these numbers and it should be correct for your eyes without having to go through this whole process again.

H. While, in most cases, having the condenser height as high as it will go will be best, you may need to adjust this for maximum ease of viewing. The knob for this is on the left side, under the stage. While looking through the microscope, raise and lower the condenser, and observe what happens to the light. The rheostat, located on the lower right

side of the microscope, can be used to adjust the amount of light put out by the lamp, and will need to be adjusted to a comfortable brightness depending on which objective lens you are using. Also, practice adjusting the iris diaphragm while looking through the microscope, and observe what happens to the light. Just like in a camera, a smaller aperture of the iris diaphragm will give greater depth of field (more of a 3D view), but let less light through, thus necessitating increasing the voltage to the lamp to compensate.

I. Note that the oculars are 10×, so if you are using a 4× objective, everything is magnified 40×, if you are using a 10× objective, everything is magnified 100×, if you are using the 40× objective, it's magnified 400× and if you are using the 100× oil immersion lens, it's magnified 1000×.



J. Use the mechanical stage controls to move the slide slightly to the right, left, toward, and away from you. Record in your notebook your observations of what happens to the image as you move the slide. Draw a picture of what the “e” looks like to the unaided eye and what it looks like under the microscope – is it right-side up? Do not make your drawings so small that you can't tell what they are when you go back to study for the final. It is suggested that drawings be at least ¼ to ½ page in size so that you can easily draw and see the details of what you observed. You don't have to worry about drawing a circle, but do indicate to the lower right of your drawing what magnification it represents. If you do draw a circle to give an idea of scale, be conscious of how much of it your specimen fills. For example, if it takes three of a certain kind of cell to span the circle, don't draw 20 of them. Refer to the Notebook Protocol section on how to draw illustrations.

K. Now, **WITHOUT MOVING THE FOCUS**, carefully swing the 10× objective around until it clicks into place **while watching from the side**. Watch you don't hit the stage or the slide with the lenses. Do be careful that you really do have enough clearance – watch from the side! Most modern microscopes, including these in the lab, are **paracentral** and **parfocal** meaning that a specimen which is in focus and in the CENTER of the field of view at 40×, will be more or less in focus and within the field of view at 100×, and if in the center and in focus at 100×, it will be more or less in focus and in the field of view at 400×. (On some antique microscopes, however, it may not work to focus in this way.) Use the fine adjustment to correct the focus as needed, then view and draw the letter “e” at 100×.

MAKING A WET MOUNT OF YEAST CELLS:

A. Obtain a blank slide and coverslip and check to make sure that they are clean. Place one drop of yeast solution on the center of the slide.

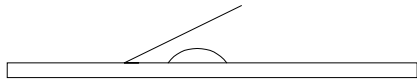


Figure 8. Side view of coverslip application

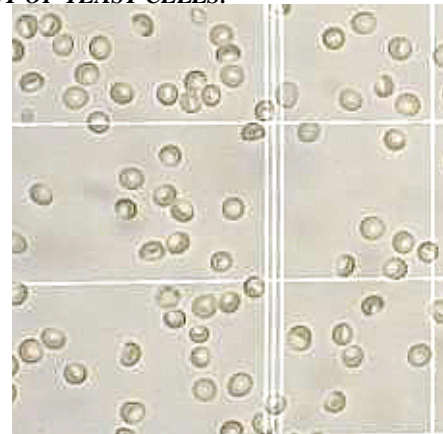
B. Do not just drop the coverslip onto the slide because large air bubbles will be trapped (note: since the yeast is alive, it may make air bubbles as it sits on the slide, but you won't have this problem with other things we will be viewing). Rather, lower the coverslip, tilted at an angle, until the lower edge touches the slide at the edge of the water drop.

C. Then, slowly lower the upper edge of the coverslip. The drop should spread out under it without air bubbles being trapped. Wipe off any excess water from the bottom, edges, etc. with a Kimwipe so the microscope doesn't get wet.

D. Examine your slide under 40, 100, and 400× (**ALWAYS START AT 40× = 4× objective**). Draw what you see at each power. Remember to make your drawings large enough. Carefully focus up and down with the fine adjustment to observe the fact that these cells are three-dimensional (adjustment of the iris diaphragm and rheostat may help you to see this better. Yeast cells should be fairly oval in shape. How much size variation can you see? Do you see any cells with reproductive buds attached? Can you see any of the organelles within the cells? Yeast cells do have a thin cell wall and clear cytoplasm. The nucleus cannot be seen unless special staining techniques are used. If you see a round object (or several) with a broad, black ring around the outside, it's an air bubble.

Switch to the 40× objective to observe the “e” at 400×. **Do not use the coarse adjustment** with the 40× objective in place – only fine adjustment should be necessary and is the **ONLY** one you should use or you could smash a lens. You may need to readjust the diaphragm and/or rheostat. Again, draw your sample as it looks at 400× and label. Practice using the controls to follow/trace the “e” at 400×. Note the individual silver grains.

L. When you are done, return to the 4× objective and lower the stage somewhat, then make sure your slide is free of fingerprints and return it to the slide box. Avoid parallax error – make sure you get the slide straight in a slot and facing the same direction as the rest of the slides.



E. Wash and dry your slide and coverslip. A Kimwipe works well to dry without lint.

F. Make another slide of the yeast, this time with a small drop of methylene blue added. Put drops of yeast solution and methylene blue near each other, but not touching and the coverslip will cause them to mix. When adding anything like methylene blue, do NOT touch the dropper to the slide. The yeast will absorb the dye and turn blue.

G. Examine under each power and draw what you see. What difference(s) does the methylene blue make in the “visibility” of the yeast cells or their organelles? Note any other observations (for example – have all of the cells taken up the dye equally?).

H. Wash (and dry) your slide and place in the designated plastic test-tube rack to completely dry. Do not store damp slides in contact with each other or they will bond tightly together and be impossible to separate when dry. Coverslips may be disposed of – do NOT leave them lying around the lab.

STORAGE OF THE MICROSCOPE:

A. Turn the rheostat down to the lowest number, then make sure the light is off. Wind the cord **NEATLY** and securely around the bottom of the arm, tucking in the end, then fully lower the stage to help hold it in place. The 4× lens should be in place below the optical head and the mechanical stage should not be sticking out on the right side (use the control knobs to move it to the center).

B. Clean up all spills on the microscope.

Proper Storage of Microscope	
1.	Turn rheostat down to #1 and turn off light.
2.	Thoroughly clean off any immersion oil and spills.
3.	Rotate 4× objective until clicked into place.
4.	Mechanical stage should not be sticking out to the side.
5.	Return diopter adjustment on left ocular to 0.
6.	Wind cord neatly in the right direction and tuck end to secure.
7.	Tighten loose screws under mechanical stage.
8.	Lower the stage.
9.	Replace dust cover in correct configuration.
10.	Place in cupboard with arm facing toward you.

C. Place the dust cover over the microscope, making sure that the numbers on the cover and microscope match (Do you have

the right cover?). Double check to make sure you have done all of these things before putting away the microscope.

D. Carefully put away the microscope with the arm of the microscope toward the door of the cabinet (so the arm can conveniently be grasped) and clean up anything else in your area. Make sure all slides have been returned to the proper place and all trash is removed. The drawer and microscope cupboard are NOT trash receptacles!



E. Report any problem with the instrument to your instructor **IMMEDIATELY**.

V. DATA:

Record all observations and data. Draw pictures and/or take notes where needed. Especially, to help your powers of observation, draw and label your own picture of the microscope – you see more when you have to draw it yourself. Also include

VI. CONCLUSIONS:

In your discussion, you should include:

1. In general, what can you say about the image you see in a microscope as compared to the direction you specimen is really facing and the direction you move the specimen?

drawings of the letter “e” as it looks to the naked eye and under the various powers (label these drawings as to which is which), a group of yeast cells under 40 and 100×, and detail of one good representative yeast cell under 400×.

2. Were you surprised to see what the yeast looked like? Did the methylene blue help you to see the yeast better?