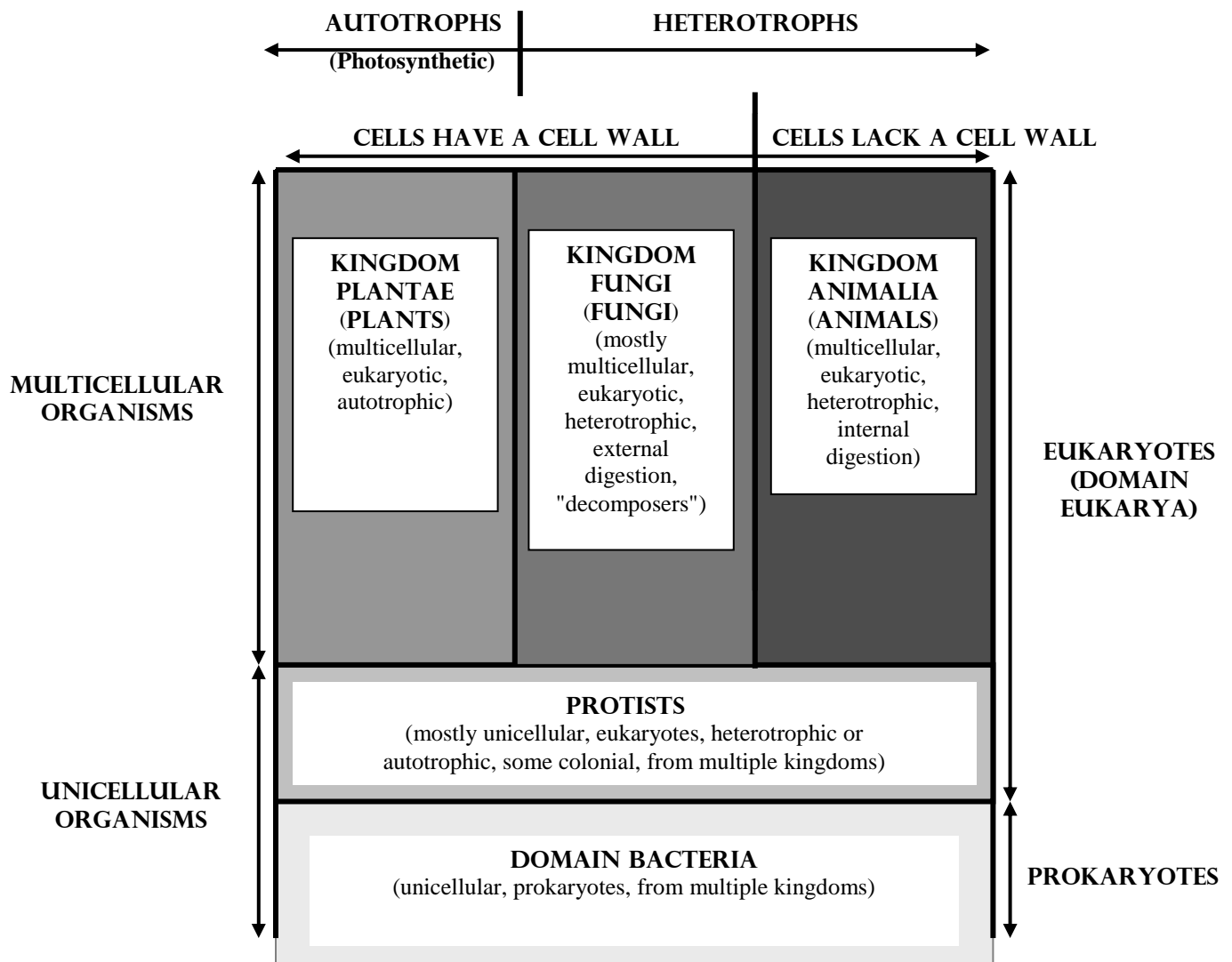


Name _____ Instructor _____ Lab Section _____

<p>Objectives: To gain an understanding of how to:</p> <ul style="list-style-type: none"> • Correctly use the compound light microscope • Differentiate between various cell types 	<p>Background material may be found in</p> <ul style="list-style-type: none"> • Chapter 1.3, 1.6 • Chapter 4.1 - 4.4 • Chapter 16 <p><i>Biology: Concepts & Connections, 8th ed.</i></p>
---	---

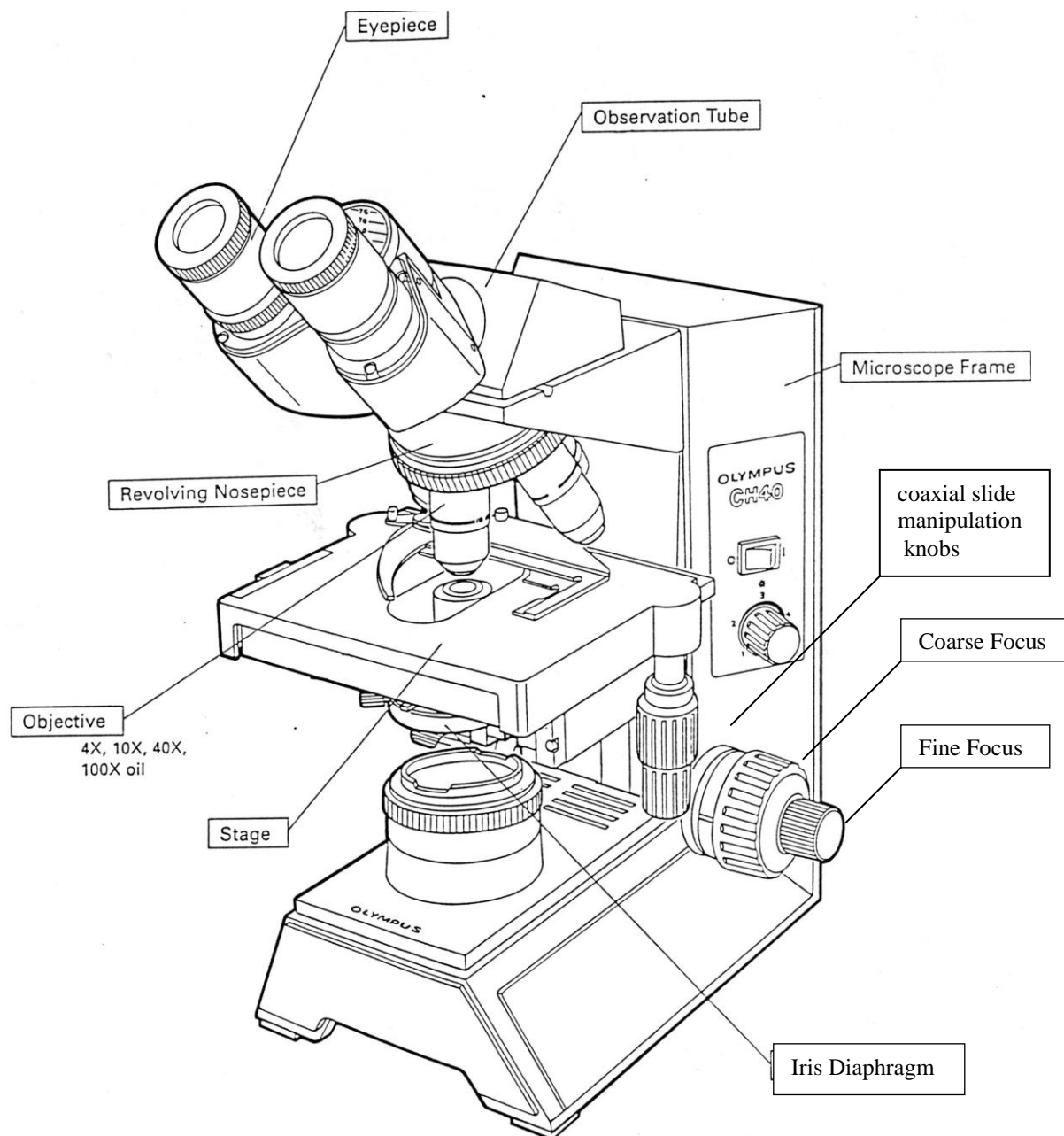
Scientists use many different tools while studying biology. One of the most powerful tools in the study of biology is the light microscope. The light microscope allows you to observe, in fine detail, the architecture of cells. A focus of these and our future studies will be the cell theory and the concept that all living things are composed of cells. Cells exist in many different shapes and sizes. Using the light microscope, we will observe the structure and behavior of a variety of cells that include representatives from two of the three Domains of Life, Eukarya and Bacteria. Eukaryotes (Domain Eukarya) include the Kingdoms Fungi, Animalia, and Plantae, as well as a diverse collection of organisms known as Protists. Prokaryotes that may be observed in lab are from the Domain Bacteria, which includes multiple kingdoms. The basic, fundamental attributes of these groups of organisms is shown in the diagram below. This lab does not address organisms from the Domain Archaea, which includes prokaryotes that live in Earth's extreme environments.



THE MICROSCOPE

Components of the Binocular Compound Microscope

1. Obtain a compound microscope from the cabinet, being sure to hold the microscope by the arm and the base.
2. Familiarize yourself with the parts of the microscope by comparing it to the illustration below.



Note: the magnification power of each individual objective lens is written on the side of each objective lens (often in colored lettering). Typical objective lens magnification powers are: 4X, 10X, 40X and sometimes 100X.

Learning to focus

Always be aware of these points before beginning to focus the microscope on a specimen:

- Always lower the stage in its lowest position before placing a slide on the stage and start focusing with the stage in its lowest position
- Begin by focusing with the lowest power objective (4X) before changing to a higher power objective
- Only use the coarse adjustment knob to focus when using the lowest power objective (4X)
- When using the 10X, 40X or 100X objectives, only use the fine focus adjustment knob to focus

1. Turn on the **light source** and adjust to mid-range.
2. Put the **stage** at its lowest position (turning the adjustment knobs toward you will lower the stage).
3. Rotate the **low power objective (4X)** into place.
4. Place a microscope slide with your specimen on the stage, clip the slide into place and center the specimen directly under the light source. You can move the slide around the stage using the coaxial slide manipulation knobs below the stage.
5. Using the **coarse focus**, raise the stage to its highest point.
6. While looking through the **ocular lens**, slowly lower the **stage** using the coarse focus until the image becomes sharp.
7. Rotate the 10X objective into place. (If you see nothing then you may need to go back to 4X and center the specimen in the field of view)
8. Refocus the specimen **using the fine focus**. (The objective lenses are parfocal and thus only minor adjustments are required to refocus when moving from one objective lens to another.)
9. Fully close the **iris diaphragm**. Slowly open it until you obtain a light intensity that is comfortable for your eyes. Note, adjustment of the iris diaphragm can result in an increase or decrease in contrast and depth of field. Very often a better result and greater contrast occurs when the light intensity is decreased (by closing down the iris diaphragm).
10. Rotate the **high power objective (40X)** into place and slowly refocus **using the fine focus**. **Note, be sure to avoid large movements of the stage while the 40X or 100X lens are in position. These lenses are very close to the slide. Both lens and slide can be damaged if allowed to contact each other.**

Magnification

 What is the magnification power of . . .

- | | |
|--|--|
| 1. the ocular lens (eyepiece)? _____ | 4. the high dry power objective ? _____ |
| 2. the low power objective ? _____ | 5. the oil immersion objective ? _____ |
| 3. the medium power objective ? _____ | |

The **total magnification** of a microscope is the magnification of the ocular lens multiplied by the magnification of the objective lens being used.

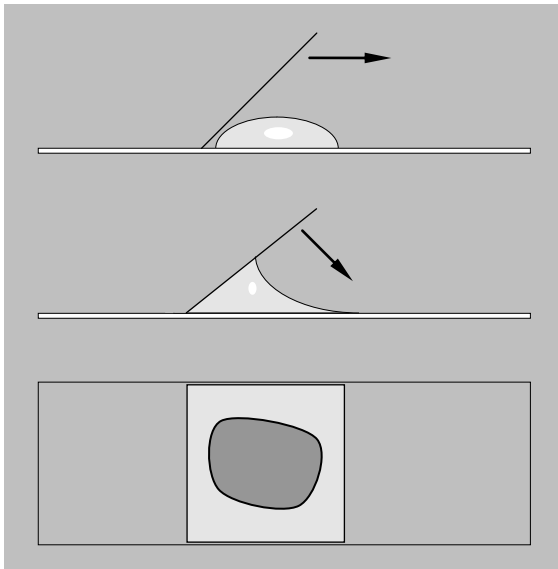
6. What is the total magnification when the low power objective is in place? _____
7. What is the total magnification when the high power objective is in place? _____

Measuring the Field-of-View: Optional as per instructor's directions

1. Rotate the 4X objective into place.
2. Place a transparent ruler on the stage and focus on its edge.
- ✎ 3. The distance between each line is 1 mm. What is the diameter of the **field of view** (the diameter of the circle of light which you see under the microscope)?
4. Rotate the 10X objective into place and focus on the edge of the rule. What is the diameter of the field of view?
5. What is the relationship between magnification and diameter of the field of view (with increasing magnification, what happens to the size of the field of view)?

VIEWING LIVE SPECIMENS

Red onion epidermis



1. Slice a red onion, separate the rings and carefully cut a 1 x 2 cm rectangle from a single layer. One side should be purple.
2. Add a drop of water to a slide. Peel the thin, nearly transparent, purple epidermis from the rectangle of onion and place it purple side up on the drop of water.
3. Add a second drop of water on top of the onion epidermis and place a coverslip on top by first touching it to the edge of the water drop and then lowering it without trapping air. Use a tissue to wick away excess water.
4. Using low power (4X), focus on your specimen and scan it by moving the slide with the coaxial slide manipulation knobs.
5. Sketch the pattern of cells observed using the 10X and 40X objectives. Estimate the size of individual cells based on the diameter of the field of view.

10X OBJECTIVE LENS



40X OBJECTIVE LENS



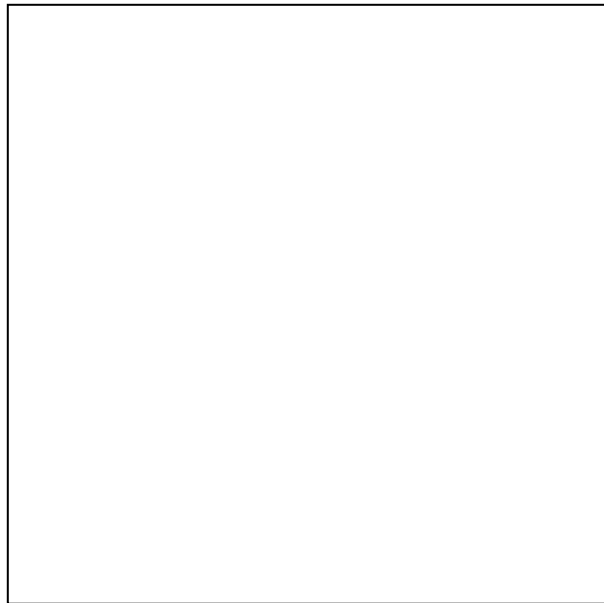
✎ CELL SIZE _____

RED ONION CELLS

Human cheek cells

1. Lightly run the wide, blunt end of a toothpick between your lower lip and teeth. By doing this you have collected hundreds of loose cheek cells from your mouth.
2. Rub a sample of the cheek cells onto a slide, and cover with a drop of 0.9% sodium chloride (this is an isotonic saline solution).
3. Add a small drop of methylene blue to the sample, and lower a coverslip on the sample and fluids, avoiding bubbles.
4. Under low power (4X) adjust the light with the iris diaphragm until the cells are visible.
5. Draw a stained cheek cell under a total magnification of 400X. Estimate the size of individual cells based on the diameter of the field of view from your previous observations. You will need to estimate cell size while using your 4X or 10X objective, then increase magnification for your drawing.

40X OBJECTIVE LENS



 **HUMAN CHEEK CELLS** CELL SIZE _____

QUESTION 

What is the key structural feature, found even in plant cells that lack chlorophyll, that could help you differentiate between plant and animal cells when viewed under the microscope?

WE WILL NOT REUSE CHEEK CELL SLIDES.

DISPOSE OF YOUR CHEEK CELL SLIDE & COVER SLIP IN A SHARPS CONTAINER.

MICROSCOPIC ORGANISMS 

Several samples of water collected from a wide variety of habitats will be available for observation with the microscope. Note the locations from which the samples were collected and prepare wet mounts of several (your instructor will instruct you as to the minimum number of samples which you must observe). Draw and label the most abundant organism from each sample you observe. Make notes on method of motility, size, color, name of the organism (if known) and indicate the objective lens you used for magnification. If you wish, you may estimate the size of the organisms based on the diameter of the field of view.

For each organism use the highest power objective possible while keeping the organism in the field of view. Draw and label (if known) as much detail as possible.



Sample from which organism was taken: _____

Species/Common Name of Organism (if known): _____

Multicellular or Unicellular? _____

Eukaryote or Prokaryote? _____

Bacteria, Protist, Plantae, Fungi, or Animalia? _____

Type of Locomotion (how does it move around?): _____

Size of Organism: _____ Objective lens used: _____

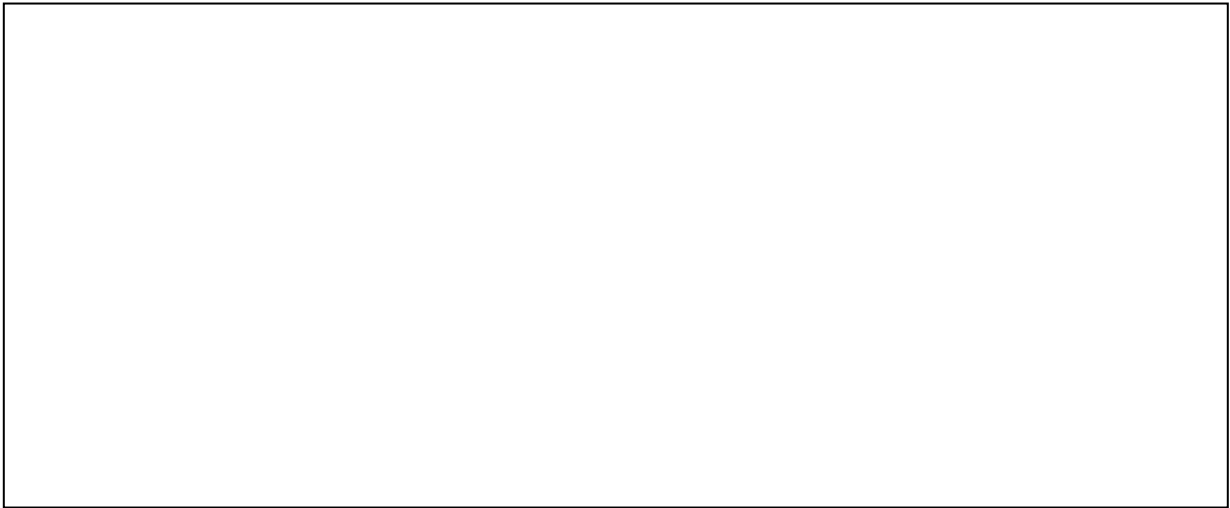
Other Notes: _____

Sample from which organism was taken: _____
Species/Common Name of Organism (if known): _____
Multicellular or Unicellular? _____
Eukaryote or Prokaryote? _____
Bacteria, Protist, Plantae, Fungi, or Animalia? _____
Type of Locomotion (how does it move around?): _____
Size of Organism: _____ **Objective lens used:** _____
Other Notes: _____

Sample from which organism was taken: _____
Species/Common Name of Organism (if known): _____
Multicellular or Unicellular? _____
Eukaryote or Prokaryote? _____
Bacteria, Protist, Plantae, Fungi, or Animalia? _____
Type of Locomotion (how does it move around?): _____
Size of Organism: _____ **Objective lens used:** _____
Other Notes:

Sample from which organism was taken: _____
Species/Common Name of Organism (if known): _____
Multicellular or Unicellular? _____
Eukaryote or Prokaryote? _____
Bacteria, Protist, Plantae, Fungi, or Animalia? _____
Type of Locomotion (how does it move around?): _____
Size of Organism: _____ **Objective lens used:** _____
Other Notes: _____

Sample from which organism was taken: _____
Species/Common Name of Organism (if known): _____
Multicellular or Unicellular? _____
Eukaryote or Prokaryote? _____
Bacteria, Protist, Plantae, Fungi, or Animalia? _____
Type of Locomotion (how does it move around?): _____
Size of Organism: _____ **Objective lens used:** _____
Other Notes: _____



Sample from which organism was taken: _____

Species/Common Name of Organism (if known): _____

Multicellular or Unicellular? _____

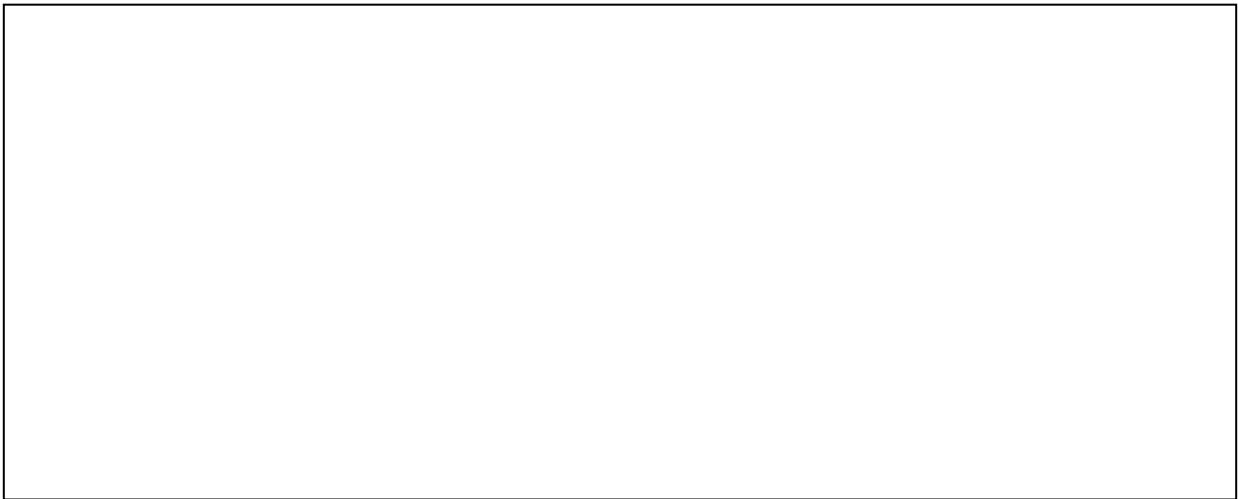
Eukaryote or Prokaryote? _____

Bacteria, Protist, Plantae, Fungi, or Animalia? _____

Type of Locomotion (how does it move around?): _____

Size of Organism: _____ **Objective lens used:** _____

Other Notes: _____



Sample from which organism was taken: _____

Species/Common Name of Organism (if known): _____

Multicellular or Unicellular? _____

Eukaryote or Prokaryote? _____

Bacteria, Protist, Plantae, Fungi, or Animalia? _____

Type of Locomotion (how does it move around?): _____

Size of Organism: _____ **Objective lens used:** _____

Other Notes:

Clean-up:

_____ **If you have not already done so, put your cheek cells slide and cover slip in a sharps container**

_____ **Rinse and dry all other slides (no soap) and return to tray on side counter.**

_____ **Put “used” coverslips in the sharps containers.**

_____ **Put “used” toothpicks in the trash.**

_____ **Return all supplies to correct trays on the side counter.**

_____ **Compound scopes:**

Turn light intensity to “1”.

Turn light switch off.

Move stage to lowest “down” position.

Place objective on low power (x4).

Unplug and wrap cord per instructor’s demonstration.

Scopes remain on table.

LABORATORY NOTES