

Microbiology for the Health Sciences Laboratory

Lab Session 1 - Microscopy

Introduction

Basic microscopy is a fundamental tool for the microbiologist. However, because our subjects are so small, we not only need to magnify them, we also need to achieve optimum resolution of our specimens. The purpose of this lab is to develop the proficiency necessary to use the brightfield microscope skillfully. While improving our microscopy skills, we will take the opportunity to begin to study the diversity of microscopic organisms in the biosphere.

A. Review the following for reference:

1. Introduction - You are responsible for following the safety precautions as they are presented! You will not be allowed in lab without a lab coat (e.g. an old shirt). The shirt must be left in lab and must not be a pull-over type t-shirt or sweatshirt! You may be given an 'F' for the lab if you purposefully do not follow safety precautions (e.g. drinking or eating in lab).
2. Text: Chap 1 - Pgs 8-14; Chap 3 – Pages 40-46; Chap 10 - Pgs 238-242, Chap 11 & 12 (look at the pictures).
3. Pay particular attention to the following: Metric units, wavelengths of light, magnification, resolution, components of light microscope (illumination source, condenser, and ocular and objective lenses), and types of light microscope (brightfield, darkfield, phase and fluorescence).

B. Do the following during lab:

1. Practice using the light microscope: Proper alignment & illumination, focusing and use of the oil immersion lens.
2. Observe microorganisms on the prepared slides: Observe, draw, describe, and classify the organisms into their proper kingdoms. Eucaryotes should be observed at 100X or 400X. Use your imagination when describing them. Procaryotes must be observed at 1000X. Descriptions of procaryotes should include: Color, Size (approximate), Shape and Arrangement of cells.
3. Observe the prepared slides set up on the demonstration microscopes: Observe, draw, describe, and classify the organisms into their proper Kingdoms.

C. Techniques:

1. Microscopy: To align the microscope for optimum resolution:
 - a. Turn on the light source.
 - b. Place a slide on the stage.
 - c. Select the 10X objective.
 - d. Adjust the intensity of light with the sliding bar.
 - e. Use the coarse, and then the fine focus knobs to focus the specimen.
 - f. Close down the sub-stage iris diaphragm.
 - g. Move the condenser up or down to focus the light on the specimen.
 - h. Use the set screws to move the beam of light into the center of the field.
 - i. Open the sub-stage iris diaphragm to just fill the field of view with illumination.
 - j. Adjust the condenser to match the numerical aperture of the objective.
 - k. Adjust the eyepieces to match your particular vision.
 - l. Repeat steps 'd' through 'j' every time you change objectives.
2. Microscopy: To change magnification:
 - a. Focus on a specimen using the 10X objective.
 - b. Move the 40X objective into position.
 - c. Use only the fine focus knob to adjust proper focus.
3. Microscopy: To use oil immersion lens:
 - a. Focus on the specimen using the 10X and then the 40X objectives.
 - b. Place a drop of oil on the slide and move the 100X objective into position.
 - c. Focus using only the fine focus.
 - d. When finished, clean the objective using lens paper.
 - e. Permanent slides can be cleaned with tissue paper.

D. Results

1. Record the observations and classifications on the observed slides in your notebook.
2. Consider organizing your notebook (a small 3-ring binder?) as follows:
 - Lab handout (with notations and directions),
 - Data (carefully organized) in tables or charts,
 - Discussion (a summary of what you did and thoughtful commentary on what it means).

Prepared Slides

1. Fungi (Note: There are three different fungi on the slide. Draw each and describe each at 100x.)

Note in particular the spore structures.

Kingdom: _____

2. Diatoms (Note: There are many types of diatoms present. Draw and describe several types at 100x.)

Note that these are single celled eucaryotes.

Kingdom: _____

3. *Volvox* sp. (Note: Draw and describe at 100x.)

Is this single or multicellular?

Kingdom: _____

4. *Spirogyra* sp. (Note: This organism is in the process of conjugation. Draw and describe at 100x.)

Observe conjugation and the spiral chloroplasts.

Kingdom: _____

5. *Spirillum* sp. (Note: This organism is small but has a precise anatomy. Draw and describe at 400x.)

Observe the flagella.

Demonstration Slides

1. Bacteria-Yeast-Blood (Note: Observe at 1000x, draw all three cell types and comment on size.)

Kingdoms: Blood _____ Yeast _____ Bacteria _____

2. *Trypanosoma gambiense*

Note the blood cells and the agent.

Kingdom: _____

3. *Giardia lamblia* (cyst stage).

Note the distinct morphology of the stage of the agent. Kingdom: _____

4. *Giardia lamblia* (trophozoite stage)

Note the distinct morphology of the stage of the agent.

5. *Trichinella spiralis*

Note the size and the internal (round) structures. What are they? Kingdom: _____

6. Liver flukes

E. Discussion and Conclusions