Background

Microbiology is study of microorganisms (microbes) and viruses. Microorganisms and viruses are too small to be seen with the naked eye. Therefore, we must use an important tool called the microscope to view microbes.

There are two main types of microscopes: the **light** microscope and the **electron** microscope. We will be focusing on light microscopes in this lab exercise. The most common types of light microscopy are **bright-field**, **dark-field**, and **phase-contrast**. We will be using the bright-field (compound) microscope. It is called a compound microscope because it magnifies through two separate lens systems: the **eyepiece** and the **objective**. The bright-field microscope works at its best when the specimen is colored or stained. This allows for us to view the cellular morphology, cell arrangements and the results of diagnostic stains.

Parts of A Microscope

Stage is a platform used to hold the slide containing the specimen. On the stage, there are mechanical clips to hold the slide in place when viewing.

Beneath the stage is the **condenser** and **iris diaphragm**. The condenser focuses light onto the specimen. Iris diaphragm can be used to adjust the amount of light emitted.

The **light source** or **illuminator** can be found at the base of the microscope. This provides light to view specimen.

The **ocular** lens is located in the eyepiece. The **objective** lens are located in the nose piece. The microscope that we will be using is equipped with three objectives: **the low-power objective**, **the high-power objective**, and **the oil-immersion objective**. To obtain the **total magnification** of a microscope, you would take the product of the ocular and objective lens used.

Basic Principles of the Microscope

The magnification of a microscope is not as important as its resolution. **Resolving power** or **resolution** of a lens is its ability to show two closely adjacent objects as distinct, discrete, and separate entities. Therefore, the largest image a microscope can produce is not actually the most useful.

Numerical aperture is a mathematical expression that describes the way in which light is concentrated by the condenser and collected by the objective or its

ability to gather light. Therefore, to get proper resolution, you need the correct amount of light to view specimen.

The **immersion oil** is used to reduce refraction or the amount of light lost. Immersion oil has the same refractive index as glass so it reduces the amount of light lost.

Parfocal is when you can switch from one power to the next and you would only need to slightly fine focus with fine-adjustment knob to get specimen into view.

Use and Care of the Microscope

1. Do not touch the lenses. If they become dirty, please use lens paper and wipe them gently.
2. When using the immersion oil, use lens paper to wipe the oil off the oil-immersion objective lens after use. Do not allow any oil to touch the other objective lens.
3. Always carry the microscope with one hand under the base and the other hand on the arm of the microscope.

Cellular Morphology

We will be using the bright-field microscope to view cellular morphology (cell shape) or arrangement of prokaryotes and eukaryotes.

Prokaryotes have no nucleus, no membrane bound organelles and are small. Eukaryotes have a nucleus, membrane bound organelles and are larger than prokaryotes. We will be looking at examples of each.

Morphology is the cell shape. There are three types: coccus (spheres), bacillus (rods), and spirillum (spiral). The morphology can be arranged in a staph, strep or single arrangement. The staph arrangement would be clusters. The strep arrangement would be chains.

For this lab exercise, we will be viewing the following specimens:

Eukaryotes

Schistosoma mansonni (40 X)
Peridinium (40X)
Trypanosoma gambiensae (mixed with RBCs; look under 100X)
Penicillium (Mold types slide purple; 40X)
Rhizopus (Mold types slide pink; 40X)
Aspergillus (Mold types slide green; 40X)
Yeast (100X)

Prokaryotes
Bacterial coccus (100X)
Bacterial bacillus (100X)
Spirillum (100X)

Protocol:

1. Using the PowerPoint provided and background information, identify all the parts of the microscope.
2. Place the slide on the stage and be sure to clamp it down with the stage clip. Use the course focus to bring the specimen up towards the objective lens. Remember to start focusing all slides under 10x first to find the object, then center the specimen and go to the 40x next, and then go to 100x if necessary.
3. For each specimen, be sure to sketch on your lab report what you observed.