Staining Bacteria

A. Introduction

Bacteria are too small to see without the aid of a microscope. While some eucaryotes, such as protozoa, algae and yeast, can be seen at magnifications of 200X-400X, most bacteria can only be seen with 1000X magnification. This requires a 100X oil immersion objective and 10X eyepieces..

Even with a microscope, bacteria cannot be seen easily unless they are stained. The procedure below should provide bacteria that can easily be seen at 1000X magnification. With this stain you should be able to see the shape of the organism, the relative size, and how variable in size and shape the bacteria are.

Choice of organisms
The larger the organism the easier it is to see. Yeast cells are large enough to be seen at 400X magnification. The largest bacteria, such as *Bacillus megaterium*, can also be seen at 400X. Most other bacteria require 1000X magnification. Algae and protozoa are best viewed in a wet mount without staining.

B. Materials

clean glass slides
culture of *Bacillus megaterium*, other bacteria, or yeast

- agar plate OR
- broth culture

inoculating loop for broth culture
toothpick for agar culture
bunsen burner
crystal violet stain
water
paper towel
marking pen
clothes pin or slide holder
250-ml beaker

C. Preparation of slide

From a culture on an agar plate

1. Label the slide so that you know which side has the bacteria.
2. Place one drop of water on the center of the slide.
3. Use the narrow end of a toothpick to remove a tiny amount of cells from the plate. You need only a barely visible amount. More is not better!
4. Disperse the bacterial cells in the water drop and spread them over a circular area in the center of the slide.
5. Allow the slide to dry.
6. Holding the slide with a clothes pin or slide holder, pass the slide slowly through the flame of a bunsen burner 3-4 time to fix the bacteria to the slide. There is no need to hold the slide in the flame.

From a broth culture

1. Label the slide so that you know which side has the bacteria.
2. Place 2-3 loopfuls of broth culture on the center of the slide. (Note, you must flame the loop between each transfer if you want to keep the culture for later use.)
3. Spread the droplets over a circular area in the center of the slide.
4. Allow the slide to dry.
5. Holding the slide with a clothes pin or slide holder, pass the slide slowly through the flame of a bunsen burner 3-4 time to fix the bacteria to the slide. There is no need to hold the slide in the flame.

D. Staining

1. Place the slide (with the label on the upper surface) on the beaker.

2. Gently place a few drops of crystal violet stain on the center of the slide where you spread the bacteria.
3. Leave the stain on the slide for 30-60 seconds.
4. Briefly wash the slide with water to remove the stain.
5. Gently blot the slide dry with paper towels. Do not rub, or you will remove the stained bacteria.

E. Viewing the Bacteria

1. Do not use a cover slip.
2. Place the slide on the stage of the microscope and begin to look for stained material using a 40X objective. You will probably not be able to see much except some purple-colored material at this magnification (unless you are using *Bacillus megaterium* or yeast). However, it is much easier to find stained material at lower magnifications.
3. When you find something visible, gently move the 40X objective out of the way (without moving the slide). Place a drop of immersion oil on the slide right below the objective, where the stained material was seen.
4. Gently move the 100X objective into the oil, without disturbing the slide. Look through the microscope. You should see the stained material, but it will probably be out of focus.
5. Slowly focus using the fine adjustment until the stained cells come into focus. You may have to move the slide slightly if the stained cells are at the edge of the field.
6. One you have the slide in focus, you can move to other fields to see more of the bacteria.
7. Clean the oil off the objective with lens paper when you are finished viewing the slide.
8. You can gently remove the oil from the slide and keep the slide for future use.