

Negative Staining

Bacteria are colorless and transparent and very difficult to observe under the microscope without some type of staining procedure. In this laboratory, we will use a negative stain procedure to observe microorganisms under the microscope.

The negative staining procedure using either nigrosine or India ink as a stain. Which is a better stain?

How does the negative staining procedure work?

In the first exercise, we will be staining a culture of known bacterium, *Bacillus megaterium*. For this exercise, refer to the Figure 10.9.

Materials needed:

- 2 slides
- Nigrosine
- Culture of *Bacillus megaterium*
- Inoculating loop

Exercise 1. Negative staining of *Bacillus megaterium*

1. Place a drop of nigrosine near the end of the slide
2. Using aseptic technique described in Laboratory 7, p. 35, dispense the organisms in the drop of nigrosine.
3. Using the second slide, spread the suspension on the slide by dragging the second slide through the drop. Your smear should be thick on one end and thin on the other. Somewhere in between should be optimal conditions.
4. Let slide dry.
5. View slide on microscope, first using scanning power, then low power and then the high power objectives. DO NOT use the oil immersion lens.

What is the shape of *Bacillus megaterium*?

What are some of the advantages of negative staining?

What are some of the disadvantages of negative staining?

Materials needed:

- 2 slides
- Nigrosine
- sterile toothpick

Exercise 2: Oral microorganisms.

1. Place a drop of nigrosine near the end of your slide.
2. Using a sterile toothpick, remove a small amount of material from between your teeth. **DO NOT** gouge your gums but gently scrape between your teeth.
3. Mix this material with the drop of nigrosine and break up any clumps. Be sure to dispose of the toothpick in a disinfectant container.
4. Spread stain on slide with a second stain as done in exercise 1.
5. Let the slide dry.
6. View microbes on the microscope. Again, use the high dry objective and **NOT** the oil immersion lens.

What shapes do you observe?