NSt Negative Staining

Learning Objectives

The student will

- Use aseptic techniques.
- Follow oral and written instructions and manage time in the lab efficiently.
- Apply correct terminology regarding microbiological techniques, instruments when making observations.
- Use the bright field light microscope to view microbes under oil immersion, make accurate observations and appropriate interpretations and store the microscope according to lab procedures.
- Describe the chemical basis for simple staining and negative staining.

Background/Theory

Dyes are selected for staining based on the chemical properties of the dye and the specimen being observed. In most cases, it is preferable to use a **positive stain**, a dye that will be absorbed by the cells or organisms being observed, adding color to objects of interest to make them stand out against the background. However, there are scenarios in which it is advantageous to use a **negative stain**, which is absorbed by the background but not by the cells or organisms in the specimen.
Negative staining produces an outline or silhouette of the organisms against a colorful background (OpenStax CNX, 2018)

Negative staining uses **acidic** dyes, molecules which when dissolved form H^+ and a negative chromogen. The negatively charged chromogens are repelled by negatively charged cell walls, leaving the cell unstained, surrounded by a colored background. Commonly used acidic dyes include acid fuchsin, eosin, nigrosin and rose bengal. (OpenStax CNX, 2018)

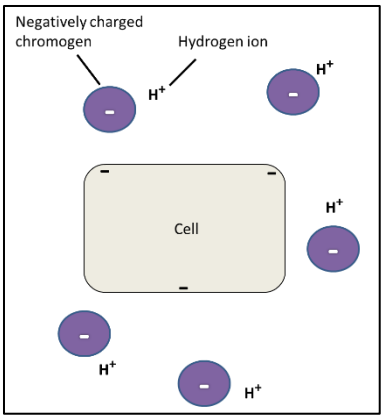


Figure 1 An acidic stain is composed of a hydrogen ions and a negatively charged chromogen.

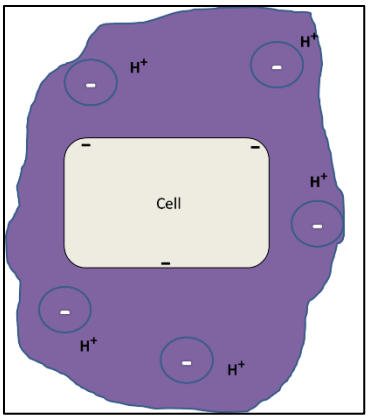
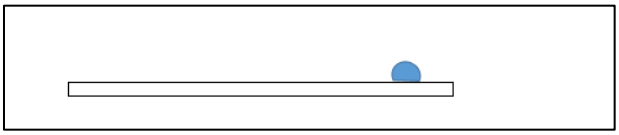


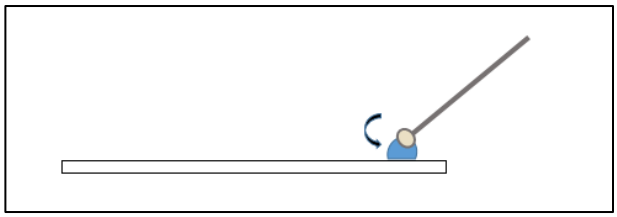
Figure 2 Result of an acidic stain. The cell is left colorless against a dark background.

In the negative staining method, a drop of the acidic stain is placed on the slide and cells growing on solid medium are mixed into it. The stain is spread thinly across the slide using a second slide, then allowed to dry. There is no need for heat fixing which can shrink and distort cells. The drying process kills most cells but not all. Therefore, be very careful to dispose of the spreading slide in the designated container immediately after use. We will refrain from using this technique with pathogenic organisms as an added precaution.

Negative Staining Procedure

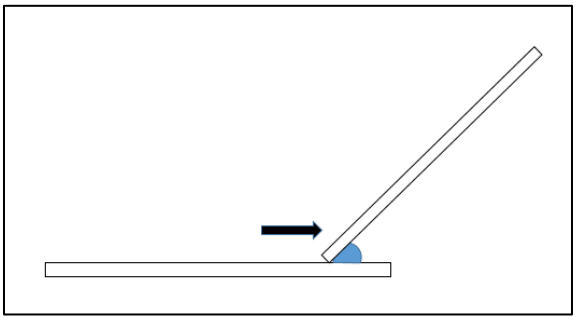
1. Place a slide on a paper towel.
2. Place one drop of an acidic stain at one end of a slide.

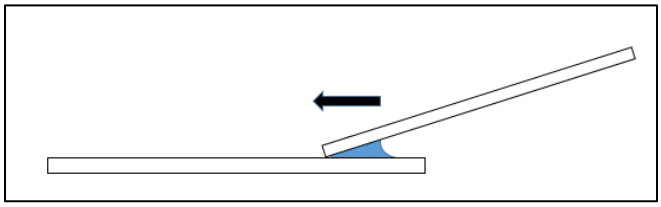




3. Aseptically obtain some cells growing on a plate or slant by touching the growth with a sterilized inoculating loop.
4. Mix the cells into the drop of stain in a circular motion being careful not to flick any liquid around.
5. Incinerate the loop and set it down.

6. Place the narrow side of a second slide up against the drop of stain so that it makes contact with it and the drop spreads out along the edge of the second slide.





7. Spread the stain across the surface of the slide, holding the second slide at an angle.
8. Immediately dispose of the second slide in a designated container.

9. Allow the first slide to dry completely before viewing under the microscope.
10. Dispose of the paper towel in the disposal container for contaminated paper.

Experiment/Exercise

Materials per student pair

4 microscope slides
Nigrosin stain
Paper towels

Cultures

Plate or slant cultures
Micrococcus luteus
Bacillus cereus

Procedure Lab 1

1. Working with your partner make one slide of each of the organisms.
2. Using both microscopes, locate the cells under oil immersion and observe.

3. Draw a representative group of cells and record the color of the background and the cell, morphology and arrangements of the groupings that you drew. Be sure to use the technical terms given in the Simple Staining exercise if applicable. Have the instructor verify that you drew what you saw by initialing the data table.
4. You make your observations directly from a microscope. Do not copy from your partner's Data and Observations sheet (nor from that of any other student).
5. You will also compare the relative cell size of a microbe prepared with a simple stain to a sample of the same microbe prepared with the negative stain. There will be a demonstration slide set up with a simple (basic) stain of *Bacillus cereus*. Note the relative length of a single *B. cereus* cell using the ocular micrometer along the pointer in the demo scope by counting the number of hash marks. Note the relative length of the *B. cereus* stained with nigrosin the same way. Because all the microscopes are the same brand and all have the same micrometer installed, we can compare the sizes of the cells without making an absolute measurement.
6. Alternatively, this exercise may be completed as a demonstration. If this is the case, make your observations from the demonstration microscopes. Take care to only use the fine adjustment. Do not move the slide. If you believe a slide has been moved or cannot focus, inform the instructor.

NSt

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Lab Report: Negative Staining

Name _____
Lab Section _____

Instr Initials

Data and Observations

Organism	Stain used and color	Drawing (See SSt, Cell Drawings.)	Morphology and Arrangement	Relative length (hash marks)
Micrococcus luteus				N/A
Bacillus cereus				
B. cereus	Basic stain	N/A	N/A	

Post Lab Questions

1. Is there an apparent size difference between *B. cereus* stained with the simple staining procedure compared to *B. cereus* stained with the negative staining procedure? If so explain.

2. What staining technique (simple positive stain, negative stain or differential stain) can be used to give accurate results for the following? (The answers may include 0-3 of the possibilities listed.)
   a. The most prevalent cellular morphology

   b. The most prevalent cellular arrangement

c. Colony morphology

d. Cell dimensions

e. The presence or absence of certain cellular structures (endospore or cell wall structure for example).

3. What staining technique (simple stain, negative stain or differential stain) can be described by each statement? (The answers may include 0-3 of the possibilities listed.)
   a. Uses basic stains

   b. Uses stains with positively charged chromogens

   c. Uses more than one stain/reagent.

   d. Requires heat fixing.

   e. Uses acidic stains.

   f. Uses stains that have H^+ as the counter ion.

References

OpenStax CNX. (2018, Mar 19). OpenStax Microbiology. Retrieved from http://cnx.org/contents/e42bd376-624b-4c0f-972f-e0c57998e765@4.24