Laboratory Resource Guide

LABORATORY EXERCISES IN MICROBIOLOGY

Tenth Edition

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Note to Instructors: The answers given for the review questions in the laboratory manual may, in many cases, not be the only possible answers or interpretations diligent students might formulate. Therefore, my objective is to indicate what answers are most likely to be given by the students.

Laboratory Report 1 Bright-Field Light Microscope (Dark Image, Brighter Background) and Microscopic Measurement of Organisms

ASSESSMENT: Critical Thinking and Learning Outcomes Review

1. **Differentiate between resolving power and magnifying power of a lens. What is meant by the term "parfocal?"**
   a. Resolving power of a lens—refers to the smallest distance between two adjacent objects on the stage of the microscope that still allows the observer to see each object independently
   b. Magnifying power of a lens—refers to the size of the image you are observing in comparison with the object's true size
   c. Parfocal—indicates that when the objectives on the microscope are rotated to view the object at a different magnification, the object will remain in focus

2. **Why is the low-power objective placed in position when the microscope is stored or carried?**
   Placing the low-power objective in position will ensure that an objective will not hit the stage if the microscope is jarred. This may also allow the barrel of the microscope to be adjusted to its lowest level to facilitate more vertical clearance when storing the microscope on its shelf or in its cabinet.

3. **Why is oil necessary when using the 90ξ to 100ξ objective?**
   Oil allows a consistent optical density for the light flow, so more light from the object will reach the viewer's eye. In addition, the oil will decrease distortion due to air currents and Brownian motion.

4. **What is the function of the iris diaphragm? The substage condenser?**
   The iris diaphragm can control the amount of light passing through the object. The condenser focuses maximum light upon the object on the slide.

5. **What is meant by the limit of resolution?**
   Limit of resolution refers to the smallest object that can be distinguished clearly with a given objective. This is a function of the numerical aperture of the lens and the wavelength of light being used.

6. **How can you increase the bulb life of your microscope if its voltage is regulated by a rheostat?**
   The bulb life of your microscope can be increased by adjusting the light gradually to that required for optimal observation when using the microscope, and by turning the light to its lowest setting when the microscope is on but the student is not looking through it.

7. **In general, at what position should you keep your microscope's substage condenser lens?**
   The substage condenser lens will need to be adjusted to its appropriate setting for each objective used.

8. **What are the three bacterial shapes you observed?**
   a. Rods (bacilli)
   b. Round (cocci)
   c. Spiral (spirilla)

9. **How can you increase the resolution on your microscope?**
   Although the quality of the lens will dictate maximum resolution, the student can achieve optimal results through proper lens cleaning and proper diaphragm and condenser adjustment.

10. **In microbiology, what is the most commonly used objective? Explain your answer.**
    When observing most bacteria, due to their small size, the oil immersion objective (100x) will be necessary. This is also true for some eucaryotic microorganisms, but most fungi, algae, and protozoa can be adequately observed with 40x or sometimes even 10x objectives.

11. **In microbiology, what is the most commonly used ocular? Explain your answer.**
    The most commonly used ocular for microbial observations is 10x to allow 900x or 1,000x magnification of bacteria.

12. **If 5x instead of 10x oculars were used in your microscope with the same objectives, what magnifications would be achieved?**
    The magnifications that would be achieved would be one-half those attained with 10x oculars.

13. **Why is it necessary to calibrate the ocular micrometer with each objective?**
    For each ocular objective combination, the relative number of lines on the ocular micrometer corresponding to one line on the stage micrometer will be different.

14. **In the prepared slides, which organism was the largest?**
    In the prepared slides, protozoa, algae, and fungi will be the largest. Of the smaller organisms (bacteria), the gram-positive stained bacteria will appear larger than gram-negative bacteria of the same actual size.

15. **When identifying microorganisms, why should a wet-mount be used when making measurements?**
    Only live unstained cells will retain their true size under microscopic examination. Stained cells will shrink and not give a true accurate measurement.

16. **What is a stage micrometer?**
    A stage micrometer is a calibrated slide which is placed upon the stage of a microscope.

17. **Complete the following for the 10x objective:**
    a. 10 ocular micrometer divisions = 7 stage micrometer divisions
    b. 1.429 ocular micrometer divisions = 1 stage division = 0.01 mm
    c. One ocular micrometer division = 0.7 stage divisions = 0.007 mm

18. Complete the following on units of measurement:

	Unit	Abbreviation	Value
a.	1 centimeter	cm	10^{-2} meter
b.	1 millimeter	mm	10_{-3} meter
c.	1 micrometer	mm	10^{-6} meter
d.	1 nanometer	nm	10^{-9} meter
e.	1 angstrom	Å	10^{-10} meter

19. In summary, what is the major purpose of this exercise?

The purposes of the exercise is to learn how to correctly use the bright-field microscope and measure microorganisms.