Objective

- To demonstrate skill in proper utilization of bright field microscopes.

Introduction

One of the most valuable tools for a biologist is the bright-field microscope (also called light microscope). This instrument derives its name from the fact that light passes from a light source through a condenser and lens system to the eye of the observer in a way that produces a bright viewing field. Properly used, the bright field microscope can open up the wide and varied world of cells— a world that is otherwise invisible to the human eye. Using the bright field microscope, a biologist can determine important information about cell morphology. A good description of cell morphology should include information about the size, shape and arrangement of cells. In the absence of costly, advanced identification techniques, a description of cell morphology is often the only basis by which to distinguish between closely related single-celled organisms.

The bright field microscopes used in this laboratory are very expensive and should be treated with great care at all times. If the microscope you are assigned to is damaged or dirty when you initially receive it, report this immediately to your TA.

Laboratory Supplies

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bright field microscope</td>
<td>1/student</td>
</tr>
<tr>
<td>Prepared slides set #1</td>
<td>2/table</td>
</tr>
<tr>
<td>Lens paper</td>
<td>2/table</td>
</tr>
<tr>
<td>Immersion oil</td>
<td>2/table</td>
</tr>
<tr>
<td>Microscopic slides</td>
<td>1 box/table</td>
</tr>
<tr>
<td>Methanol bottle</td>
<td>1/table</td>
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</table>

Procedures

1. Obtain your assigned microscope from the cabinet. Be careful to avoid striking the microscope as you remove it from its storage cabinet. Grasp the spine of the microscope with one hand and support the base with the other. Carry the microscope with its plastic cover in a vertical position to your work area.

2. Make sure that the ocular and objective lenses are clean. If not, refer to steps 14-16.

3. Plug in the microscope and gently turn on the light source.

4. Mount one of the prepared slides (from the slide box supplied) on the mechanical stage. It is best for a beginner to start with the slide of color threads or the slide of letter “e”.
5. Raise the condenser to 2-3 mm from its uppermost position and open the iris diaphragm 3/4 of the way.

6. Turn the turret so that the low power (4X) objective is locked into position. The low power objective is used primarily for scanning the microscopic field and for viewing large specimens.

7. Decrease the distance between the objective lens and the slide by turning the coarse focus knob. Bring the objective lens close to the slide without touching the slide. **WARNING:** Do not turn the coarse focus knob so far that it touches or breaks the slide.

8. While looking through the ocular lens, turn the coarse focus knob to increase the distance between the slide and the objective lens. Use the fine focus knob to bring the image into sharp focus.

9. Adjust contrast by raising/lowering the condenser and/or opening/closing the iris diaphragm. Once focus and contrast are achieved, scan the slide by moving it with the stage knobs. Note that these knobs have numerical x and y coordinates which may be recorded for a given location on the slide.

10. Rotate the 40X objective into position. The microscopes used in this lab are "parfocal," which means that if the image is focused for one objective lens, it will be nearly focused for all other objectives. Therefore, you will only need to adjust the fine focus to sharpen the image. As magnification is increased, the condenser should be moved closer to the stage, and the iris diaphragm should be opened gradually until optimum contrast and resolution are achieved. Immersion oil is used if and only if the 100X objective is used. When using oil, the condenser should be in its uppermost position and the iris diaphragm should be all the way open.

11. To apply immersion oil, rotate the turret to a position halfway between the high dry (40X) and the oil immersion (100X) objectives. Place a very small drop of
immersion oil on the circle of light on the slide. Rotate the oil immersion objective into position and focus with the fine adjustment only.

12. After observing the slides of colored threads and the letter “e”, put a slide of actual bacteria on the stage and go through the same procedure. Record the shape of the organisms and make a sketch of their cell arrangement on your result sheet. Result sheets will be collected by your TA at the end of the session and will be graded. Note that on this slide, there are 3 different bacterial species smeared on the left, middle and right portions of the slide. Find each species and draw them separately.

13. When your work is finished, remove any slide that might still be on the mechanical stage.

14. Clean oil from oil immersion objective using lens paper. Do not use any other type of paper, as you might scratch expensive lenses. If you have used prepared slides, they should also be cleaned of any oil in the same way.

15. Inspect other objective lenses and stage for oil and dust. Place the 4X objective into position. Place the condenser and stage to their lowermost positions.

16. Inspect and clean the ocular lenses. Replace the plastic cover on microscope and return microscope to its assigned position in the storage cabinet before leaving the lab.

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Results of Microscopy Lab Exercise

Name ____________________________ Date _____________ Section ____

Organism Shape  Drawing

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