Microbiology Lab 3

Using the Microscope

Begin by bringing a microscope to your lab bench (one per lab group). Uncover and note all the components listed in your handout. Make sure your scope has 4 objective lenses.

1. Slide one—obtain a slide with the 3 threads on it. Use your scanning, low and high (dry) power objective lenses to view. What details can be seen at each power? Attempt to determine which thread is on the bottom, middle and top. Which objective best allows this determination? **Sketch an image from scanning or low power.**

2. Slide two—obtain a slide with a newsprint word on it. View the letters on scanning, low and high power. What detail of the paper and printed letters do you notice? How does the image of the letter "e" compare to the way it appears on the slide? Was this difference evident on the thread slide? **Sketch an image of the "e" from scanning or low power.**

3. Slide 3—obtain a slide with a micrometer ("tiny ruler") on it. View it on scanning, low and high power. How would you use the micrometer? How does an increase in magnification change the image of the micrometer ticks (lines). How does the field of view change with increasing magnification (according to your micrometer)? Use the micrometer to measure each field using different objective lenses. **Sketch a portion of the micrometer using each level of magnification.**

4. Read the handout pertaining to oil immersion use. Choose a prepared slide of *Micrococcus luteus.* Carefully find the organisms under high (dry) power (They may appear as a pink blur). Why do you suppose they appear pink? Use a drop of oil to use the oil 100X objective lens and focus with the fine adjustment. Sketch some details of your bacteria. Describe the shape and arrangement of the bacteria. Note the scientific name.

THE USE AND CARE OF THE MICROSCOPE

Most of you in this class have handled microscopes before but relatively few of you have used oil immersion microscopes with magnification as high as these.

Follow the instructor as the identity and function of the parts and controls of the microscope are explained. There are several different designs of microscopes in the class but the basic components will be similar.

Operation of Microscope

Eyepiece or Ocular. The function of the eyepiece is that of a 10 power (10X) magnifying glass which remagnifies the image formed of the specimen by the objective lens. A common reason for a blurred image is a fingerprint across the eyepiece. A good test to see if the eyepiece is responsible for fuzziness or dust particles in the image is to rotate the eyepiece. If this identifies the eyepiece as the source of the problem, use only lens paper in your lab kit to clean it.

Objective Lens. There are usually three of these on a revolving turret. These lenses are designed so that if an object is located, centered, and focused in a lower power objective, it will be visible and roughly in focus when a higher powered objective is rotated into place (parfocal). This is useful in microscopy since the highest power objective covers an extremely small field of view. The table summarizes some information about the objectives on your microscope.

Objective Lens	Objective Magnification	Total Magnification (with 10X ocular)	Numerical Aperature	Working Distance (mm)	Diameter of Field (mm)
Scanning	4X	40X	0.10		~4 mm
Low power	10X	100X	0.25	5.4	1.8
High power	40X	400X	0.65	0.39	0.45
Oil immersion	100X	1000X	1.30 (1.25)	0.11	0.18

Scanning 4X

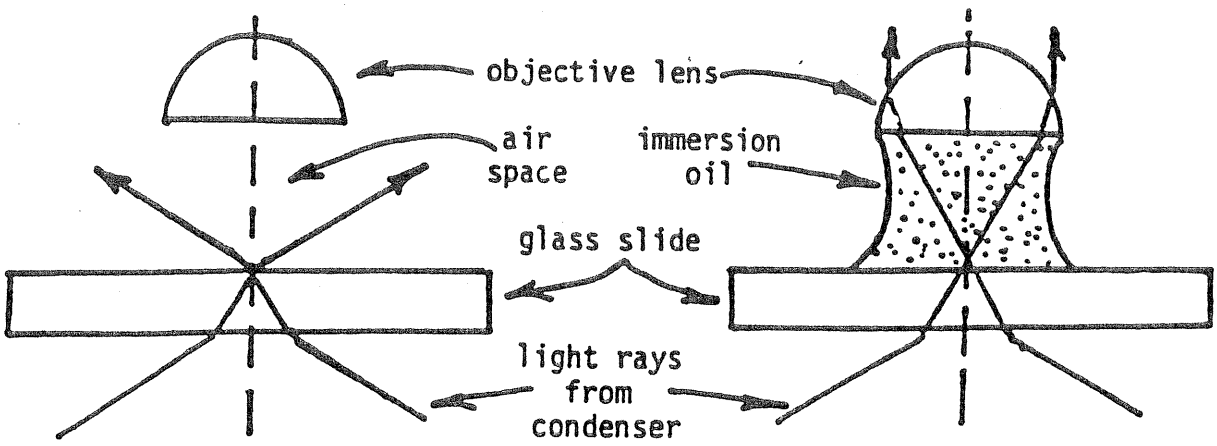
Low Power. This is usually marked 10X. However, remember that the image formed by this lens is remagnified by the eyepiece (10X) and the total magnification of the microscope is 100X. This is a common cause of confusion with students.

High Power. This is typically about 40X magnification and the total magnification is 400X.

Oil Immersion. This is the highest power objective, usually 100X magnification, making a total magnification of 1000X. This objective usually has a black band to identify it. To use it a drop of immersion oil must be placed on the microscope slide and the tip of the objective immersed in it. This oil has the same refractive index as glass.

As can be seen in the drawing below, the oil becomes a part of the optical system of the microscope. Without oil, light rays bend when leaving the glass slide into the airspace below the objective and are lost. With oil these rays are captured. This has the same effect as making the objective larger in diameter. In the equation for calculating resolving power given below this would be reflected as an increase in the numerical aperture and improves the resolving power.

To be practical about it, if you forget the oil you will have a fuzzy image with poor resolution.



Resolving power is an important consideration. It is based upon the equation shown here:

$$\text{Resolving power} = \frac{\text{Wavelength of light}}{(2) \text{ times (Numerical aperture)}}$$

The smaller the number representing the resolving power the better. It represents the ability to separate two closely spaced points as separate points without blurring them together. If the average wavelength of visible light, .540 micrometer (μm), and the numerical aperture of (N.A.) stamped on your microscope's oil immersion objective (1:30) are inserted into the equation, the resolving power calculates out as about 0.2 μm.

In practical terms a common bacterium used in this class, *Escherichia coli*, is a rod about 0.5 μm by 1.0 μm in size. Since the microscope's resolving power is about 0.2 μm, any points in the organism not separated by about half of its diameter would not be resolved as separate points. Also, organisms in close contact with other organisms tend to blend into each other and their outlines become unclear.

Therefore, when trying to determine the shape of a bacterium try to look at clearly separated cells. This is also why you cannot see bacterial flagella, which are too slender for the resolving power of these microscopes.

Another way of looking at resolving power is to consider that a person magnified 1000 times, as in these microscopes, would be on the order of a mile in height. But with equivalent resolving power, such details as feet or hands on a human would not be clearly apparent.

In your lecture text, you should be able to find examples of microbial images made by electron microscopes in which magnifications of 100,000X or even higher are used. A human under these magnifications would be something like a hundred miles tall. Note that the resolution is still

good. This is because the electron beam has a much shorter wavelength (see the equation) than visible light and permits such magnifications. The image of a light microscope could be magnified by 100,000X but the resolving power would be so poor that this is called `empty' magnification.

Coarse Focusing Knob. This knob is used for making large adjustments in focus. You will notice, particularly with the oil immersion objective, that it is almost impossible to get the image properly focused with use of this knob alone.

Fine Focusing Knob. Before using this knob, the image should be approximately in focus with the coarse adjustment. Take care in focusing downward with this knob. The gearing is such that it is easy to damage the expensive objective lens or break the glass slide. Newer microscopes have a spring-loaded nose on the objective to minimize this. However, this has the disadvantage that it is easy to have the objective in contact with the slide, focus it upwards and have it still in contact with the side -- and still out of focus.

Mechanical Stage. Note that this is designed with a spring-loaded clasp to hold the slide as it is moved about on the stage. The slide is grasped at the edges. A common student mistake, especially with the 'claw' type, is to place it on top of the slide. Manipulate the two knobs and note the movement.

Condenser. For most purposes the condenser should be positioned about as high as it will go so the upper surface of the lens is almost in contact with slide bearing the specimen. If the condenser is out of position, the image will be dark and indistinct. In this class, we do not put oil between the slide and the top of the condenser. However, students who intend to use a microscope professionally should know that if oil is not also in this position, but only between the slide and the objective, that the numerical aperture (N.A.) is limited to 1.0 instead of the marked 1.30. The resolving power is thereby decreased.

Iris Diaphragm. The condenser is provided with an iris diaphragm similar to that found on adjustable cameras. In this laboratory class, the main use is to cut down the light and get better contrast when viewing unstained organisms such as in the experiment with molds and yeasts. The adjustment is usually made by means of a lever which is usually opposite the place where you first fumble for it. Others are adjusted with the knurled ring around the condenser. Move the lever or adjust the ring and observe the variation in light.

Persons who will use a microscope professionally should know that the real purpose of the iris is to enhance image contrast by adjusting it so as to eliminate extraneous light rays from the field. This is done by closing down the aperture by about 25%.

Directions for focusing. Most people unfamiliar with focusing an oil immersion lens have best success by first locating the specimen under the low power objective and then rotating the oil immersion objective into place. With more experience it is possible to approximately center the specimen and bring the oil immersion lens down until it gently touches the slide. The glass lens of the objective is recessed in the metal and does not contact the slide. Also note that the nose of the oil immersion objective is spring loaded. If forced down it should not be damaged. However, this also means that when the spring is compressed, you might move the objective upward quite a ways without changing the focus. Then use the coarse adjustment to raise the lens as slowly as possible. Usually you will pass through the center of focus before you can stop, and a few turns down with the fine adjustment will bring the specimen into focus.

When in focus, the objective is so close to the slide that the cover of this manual would barely fit the gap (see Table). If there are no stained cells in view, they can be located by bringing the lens just barely clear of the slide and moving the slide quickly back and forth. Look for light and dark in the eyepiece and stop over a dark area, which is usually a stained area of the specimen.

The area seen through the oil immersion objective is only 0.18 mm, or about the size of the period printed at the end of this sentence.

Cleaning the oil immersion objective. If immersion oil is left on this objective it can eventually seep into the lens elements. Use lens paper, only, to wipe off the oil before returning the microscope to the cabinet.

General Microbiology
Lab 3
Oil Immersion Light Microscopy

Today we will view microorganisms using the oil objective. Recall the reason for using oil to reduce light refraction when using high magnification.

Be sure to use careful technique when using the oil immersion objective.

1. Try to position your slide so that the objective will swing into position without excessive movement of the stage. (you may be able to detect color or blurred image at lower powers)

2. Add oil to your slide with the oil objective moved out of the way.

3. Watch your stage as you swing the oil objective into position. Make sure the objective is above the oil drop and watch as you move the objective into the drop. Focus SLOWLY with the course focus and then with the fine focus.

4. If your image does not come into focus with minimal adjustment, look at the stage again. Reposition the objective if necessary and repeat step 3.

5. Take care to drop oil only on the slide. Wipe any surface before oil is spread to other parts of the scope.

6. Use only lens paper to clean the objective, when finished. Follow instructor's procedure.

7. Always return scope to cabinet with low power objective in position to prevent damage to high and oil lenses.