

Microscopy Laboratory

I. Identify the following parts of the microscope:

- oculars
- nosepiece
- objectives, stage
- condenser
- diaphragm
- illuminator, light source
- lock screw
- coarse and fine adjustment

What function does the condenser have?

What function does the diaphragm have?

II. For each objective, determine magnification, total magnification and numerical aperture

Objective	Magnification	Total magnification	Numerical Aperture
Scanning power			
Low power			
High power (high dry)			
Oil immersion			

III. Define the following terms:

- Resolution / Resolving power

- Define resolution mathematically.

- Parfocal

- Numerical Aperture

IV. Calculate the resolving power for each of the four lenses (using the equation you found for resolution above).

When calculating resolution, use 686 nm as an average wavelength for white light.

Objective	Resolution
Scanning power	
Low power	
High power (High dry)	
Oil immersion	

How do you get maximum resolution from a lens system?

V. Care of Lenses

What type of cleaning tissue should be used?

What type of solvent is considered best? What other possibilities exist for solvents?

VI. Exercise

Materials needed:

- Prepared slide of letter e
- Prepared slide of colored threads
- Prepared slide of microbes (as directed by your instructor).

Exercise 3: Slide of letter e. See procedure for set-up in your lab manual on p. 6. Be sure the objective in place on the microscope is the scanning power objective.

1. Position letter e slide on the stage as in Fig. 1.7.
2. Find the letter e under scanning magnification, focus as needed.
 - What does the letter e look like with respect to orientation when viewed without magnification?
3. Be sure the letter is in the exact center of field of view. Move up to low power and view the letter e. Focus as needed using only the fine focus adjustment.
 - Are you viewing more or less of the letter e?
4. Move up to high power, view the letter e focussing as needed with fine adjustment only!!!
 - Are you viewing more or less of the letter e?

If the letter e is not in the center of the field of view, what would happen as you move to low or high power? Try it.

When viewing the slide through the scanning objective, move the slide to the right. As you do this, check the direction the slide is moving on the stage without magnification. Which way is it moving, when viewed with the ocular and when viewed with the naked eye?

Exercise 2: Colored threads. View the colored threads slide on the microscope using the same procedure as above.

Determine the order of these threads.

Exercise 3: Microorganisms. View already prepared slides as directed by your instructor.

1. View these slides using scanning power.
2. Then move to low power and focus using the fine adjustment.
3. Then move to high power and focus using fine adjustment.
 - Can you determine shape, color or arrangement using the high dry objective?
4. Now move to the oil immersion objective by sliding the objectives to a position between the high power and the oil immersion objective.
5. Place a drop or 2 of oil on the slide and slide the oil immersion objective in place. Note: **DO NOT** place the high dry lens in the oil. This objective is not sealed to prevent oil from leaking into the objective and you will **ruin the high dry objective!!!** Also, **DO NOT** move your focus adjustment when switching from high dry to oil, your objective will NOT hit the slide!!
6. Now view the specimen and focus using the fine adjustment only. **DO NOT** use the coarse adjustment. Since microscopes are parfocal, you won't need to focus very much. If you focus one way and cannot find the organism, focus in the direction.
 - Now describe color, shape and arrangement.

Oil enhances the resolving power of the microscope--how?

VII. More on Resolution

Calculate the resolving power for the oil immersion lens using a blue filter (wavelength = 400 nm), green filter (wavelength = 600 nm) and a red filter (wavelength = 800 nm).

Wavelength	Resolution (nm)
Blue light ($\lambda = 400$ nm)	
Green light ($\lambda = 600$ nm)	

Red light ($\lambda = 800 \text{ nm}$)	
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Which light would give the best resolution?

How can you extend lamp life?

VIII. Storing microscope

- Remove the slide.
- Wipe off the objective lenses with a soft cloth. If you've been using oil, you should clean the oil off the lenses with an approved solvent.
- Be sure to clean off any oil that has dropped on the stage or other parts of the microscope.
- Slide the scanning objective into position. Putting the scope away with the oil immersion or the high dry objective may damage those lenses.
- Wrap cord loosely around the microscope. If the cord is tight, it may result in breaking the wires and causing the light to short out.
- Place vinyl cover back over the microscope to prevent dust accumulation.

IX. Types of microscopy.

Be certain that you understand the purpose of each of these types of microscopy and be able to list advantages and disadvantages of each and understand the best times to use these microscopes. Refer to Chapter 3 of your textbook and lab exercise 1-4 in your lab manual.

- Brightfield microscopy.
- Darkfield microscopy
- Phase contrast microscopy

- Fluorescence microscopy

- Electron microscopy

X. Sources:

1. Benson, Microbiological Applications: Exercises 1-4
2. Talaro, Foundations in Microbiology, Chapter 3.