Lab 4: Introduction to Microscopy

BIOL-8

Name: ______________________ **Date:** ______________________

Name: [ ] **Date:** [ ]

Objectives

By the end of this lab, you will be able to:

- **Identify the parts of a compound microscope** and describe their functions
- **Demonstrate proper microscope techniques** for focusing and handling
- **Prepare wet mount slides** for microscopic observation
- **Calculate magnification and specimen size** using appropriate formulas
- **Compare how images appear** through the microscope versus naked eye
- **Observe a variety of specimens** including prepared slides and self-made slides

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Introduction

The microscope is one of the most important tools in biology. It allows us to see structures that are invisible to the naked eye—from cells to microorganisms to the intricate details of tissues. In this lab, you'll learn proper microscopy techniques, including how to focus, calculate magnification, estimate specimen size, and prepare your own slides.

Historical Note: The first microscopes were developed in the late 1500s. In 1665, Robert Hooke used an early microscope to observe cork and coined the term "cells."

Key Terms:

- **Magnification**: How many times larger an object appears
- **Resolution**: The ability to distinguish two close objects as separate
- **Field of view**: The circular area visible through the microscope

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Part 1: Microscope Parts and Functions

> ***Learning Goal:*** *Become familiar with the components of a compound light microscope before using it.*

Microscope Diagram

Study the microscope at your station. Use this diagram as a reference and label the parts in the table below:

```
                  ╔══╗
                  ║  ║ ← Eyepiece (Ocular Lens)
                  ╚══╝
                    │
               ┌────┴────┐
               │  Body   │ ← Body Tube
               │  Tube   │
               └────┬────┘
          ╭─────┴───────╮
         ╱    Revolving    ╲
            (     Nosepiece     ) ← Holds Objectives
         ╲_______________╱
              │ │ │ │ │ │ │
          ╰─┼─┼─┼─╯ ← Objective Lenses
                │ │ │
     ┌────────────────────────────┐
     │                            │ ← Stage
     │                            │
     │    ╔════════════════╗      │
     │    ║    (Slide)     ║      │
     │    ╚════════════════╝      │
     └────────────────────────────┘
            │            │
     ╭──────╯            ╰──────╮
     │ Coarse        Fine       │
     │ Adjustment    Adjustment │
     ╰──────────────────────────╯
                 │
     ┌───────────┴──────────┐
     │                      │ ← Arm
     │  ┌──────────────┐    │
```

```
│   │   Light /    │   │ ← Light Source
│   │  Diaphragm   │   │
│   └──────────────┘   │
└──────────────────────┘
═══════════════════════ ← Base
```

Microscope Parts — Identification

#	Part Name	Location on Microscope	Function
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			

Magnification Levels

Your microscope has multiple objective lenses:

Objective Lens Magnification

#	Objective Lens	Objective Power	Eyepiece Power	Total Magnification
1				
2				
3				
4				

Calculating Total Magnification:

```
Total Magnification = Eyepiece Power × Objective Power

Example: 10× eyepiece × 40× objective = 400× total magnification
```

Part 2: The Letter "e" Observation

> ***Learning Goal:*** *Discover how the microscope inverts images and how to move specimens.*

Background

The compound microscope creates an image that is both **inverted** (upside down) and **reversed** (left/right flipped). This affects how you move slides to keep specimens in view.

Procedure

1. Obtain a prepared slide with a letter "e" (or cut a small letter "e" from newspaper)
2. Place the slide on the stage with the "e" right-side up and readable
3. Start with the lowest power objective (4× or 10×)
4. Focus carefully using coarse then fine adjustment

5. Observe and record how the letter appears

Data Collection

Letter 'e' Observations

#	How does the letter "e" appear?	Sketch what you see through the microscope
1		

Draw the letter "e" as it appears on the slide (naked eye) and through the microscope:

As placed on stage	As seen through microscope
e	

Movement Investigation

When you move the slide to the RIGHT, which direction does the image move?

When you move the slide AWAY from you (up), which direction does the image move?

Why does this happen? Explain in terms of how light passes through the microscope:

Practical implication: If a specimen you want to view is in the upper right corner of the field of view, which direction should you move the slide?

Part 3: Calculating Specimen Size

> ***Learning Goal:*** *Learn to estimate the actual size of microscopic objects using field of view diameter.*

Background

To calculate specimen size, we first need to know the **field of view diameter** at each magnification.

Key relationships:

- Higher magnification = smaller field of view
- Field of view diameter and magnification are inversely proportional

Measuring Field of View

Place a transparent metric ruler across the stage and measure the field of view diameter at low power:

How to Measure Field of View:

```
        ┌─────────────────────────┐
      /                             \
     |     ╔════════════════════╗     |
     |     ║ ← Ruler markings → ║     |
     |     ║  | | | | | | | |   ║     |
     |     ║  0 1 2 3 4 5 mm    ║     |
     |     ╚════════════════════╝     |
      \                             /
        └─────────────────────────┘
              Field of View

   Count the mm markings visible = FOV diameter
```

Field of View Diameter

#	Objective	Total Magnification	FOV Diameter (mm)	FOV Diameter (μm)	How Measured?
1					
2					
3					
4					

Step-by-Step FOV Calculation for High Power:

```
┌──────────────────────────────────────────────────────────────┐
│  FORMULA: FOV₂ = FOV₁ × (Magnification₁ ÷ Magnification₂)  │
└──────────────────────────────────────────────────────────────┘

EXAMPLE:
• Low power (100×) FOV measured = 2.0 mm
• High power (400×) FOV = ?

  FOV at 400× = 2.0 mm × (100 ÷ 400)
              = 2.0 mm × 0.25
              = 0.5 mm
              = 500 μm

YOUR CALCULATION:
• Your low power (100×) FOV = _______ mm
• High power (400×) FOV = _______ mm × (100 ÷ 400)
                        = _______ mm × 0.25
                        = _______ mm
                        = _______ μm
```

Estimating Specimen Size

Once you know the field of view diameter, you can estimate specimen size:

Example:
If the field of view diameter at 100× is 1.5 mm (1500 µm), and a specimen takes up half the field:

Specimen size = 1500 µm × 0.5 = 750 µm

Convert to different units:

- 750 µm = 0.75 mm
- 750 µm = 0.00075 m

Part 4: Observing Prepared Slides

Learning Goal: *Practice focusing and observing different types of specimens using proper technique.*

Procedure

1. Always start at the LOWEST magnification
2. Use COARSE adjustment to bring specimen into rough focus
3. Use FINE adjustment to sharpen the image
4. When changing to higher magnification:
5. Center the specimen first
6. Rotate to higher objective
7. Use ONLY fine adjustment (never coarse on high power!)
8. Adjust diaphragm for optimal lighting

Observations (Prepared Slides)

Prepared Slide Observations

#	Slide Name/ Description	Magnification Used	Sketch What You See	Estimated Specimen Size
1				
2				
3				
4				

Drawing Area

Draw what you observe through the microscope in the circles below. Include labels where possible.

Slide 1:

```
    _______________
   /               \
  /                 \
 |                   |
 |                   |
 |                   |
  \                 /
   \_________________/
```

`Magnification: _____×`

Slide 2:

```
    _______________
   /               \
  /                 \
 |                   |
 |                   |
 |                   |
```

```
  \                   /
   \_________________/
```

Magnification: _____×

Slide 3: ______

```
      _______________
     /               \
    /                 \
   |                   |
   |                   |
   |                   |
    \                 /
     \_______________/
```

Magnification: _____×

Part 5: Making Your Own Wet Mount Slides

> ***Learning Goal:*** *Learn to prepare simple wet mount slides for observation.*

Materials Needed

- Clean glass slides
- Coverslips
- Dropper and water
- Specimens (provided by instructor)

Procedure

1. Place a clean glass slide on the table
2. Add ONE drop of water to the center of the slide
3. Place the specimen in the water drop
4. Hold coverslip at 45° angle, touching one edge to the water
5. Slowly lower the coverslip to avoid air bubbles
6. Blot excess water with paper towel (if needed)

Specimens to Prepare and Observe

Wet Mount Slide Observations

#	Specimen	Magnification	What did you observe?	Estimated size	Drawing on next page?
1					
2					
3					
4					
5					

Size Estimation Exercise

Estimate the size of one of your specimens using field of view:

Specimen chosen: ____

Field of view diameter at the magnification used: ____ µm

The specimen appears to take up approximately ____ (fraction) of the field of view.

Calculation:
Estimated size = FOV diameter × fraction
Estimated size = _ **µm** × = ___ µm

Convert to mm: _ **µm ÷ 1000 =** ____ mm

Bonus Activity: The Letter on Paper

Observe newspaper print or typed text under the microscope:

What happens to the appearance of printed letters under magnification?

Can you see individual dots or ink patterns? Describe what you see:

Estimate the size of one printed period (.) or letter:

Part 6: Troubleshooting and Best Practices

> ***Learning Goal:*** *Recognize common problems and know how to solve them.*

Common Microscope Problems

Troubleshooting Guide

#	Problem	Possible Cause	Solution
1			
2			
3			
4			
5			
6			

Proper Microscope Care Checklist

Before putting away your microscope:

- [ ] Rotate to lowest power objective
- [ ] Remove slide from stage
- [ ] Clean lenses with lens paper only (never paper towels!)
- [ ] Lower stage to lowest position
- [ ] Turn off light source
- [ ] Cover microscope (if applicable)
- [ ] Return to proper storage location

Why is it important to store the microscope on the lowest power objective?

Part 7: Size Comparison Summary

> ***Learning Goal:*** *Develop a sense of scale for biological structures.*

Relative Sizes

Rank the following from smallest to largest (1 = smallest):

Specimen	Your Rank (1-8)	Typical Size
Human hair diameter		50-100 µm
Red blood cell		7-8 µm
Bacterium (E. coli)		1-2 µm
Human cheek cell		50-80 µm
Plant cell		10-100 µm
Virus particle		0.02-0.3 µm
Thread fiber		10-30 µm
Printed period (.)		~400 µm

Which of these could you see with a standard light microscope (resolution limit ~0.2 µm)?

What type of microscope would be needed to see viruses?

Conclusions

1. What was the most surprising thing you observed under the microscope today?

2. Why do we always start at the lowest magnification?

3. Explain the relationship between magnification and field of view:

4. Describe one way microscopes are used in medicine or healthcare:

5. What would you like to observe under the microscope in a future lab?

Quick Reference: Units of Measurement

Unit	Symbol	Relationship	Use
Meter	m	1 m = 1,000 mm	Large organisms
Millimeter	mm	1 mm = 1,000 µm	Small organisms, tissues
Micrometer	µm	1 µm = 1,000 nm	Cells
Nanometer	nm	1 nm = 0.001 µm	Molecules, viruses

Conversion practice:

- 50 μm = 50/1000 = 0.05 mm
- 2 mm = 2 × 1000 = 2000 μm
- 0.5 mm = 500 μm

Formula Summary

Formula	Purpose
Total magnification = Eyepiece × Objective	Calculate how much larger image appears
$FOV_2 = FOV_1 \times (Mag_1/Mag_2)$	Calculate field of view at different magnification
Specimen size = FOV × fraction	Estimate actual specimen dimensions

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Connection to Module 04: Understanding cell structure requires microscopy skills. The techniques you learned today—proper focusing, slide preparation, size estimation—will be essential for observing cell organelles, tissues, and microorganisms throughout this course. Microscopy is also a key diagnostic tool in medicine, from blood smears to tissue biopsies.

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