

Microscope and Microscopy

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Types of Microscope

- **Compound microscope**
The compound microscope is the most common of the microscopes. Essentially, a compound microscope consists of two systems of lenses, an ocular (eyepiece) in the upper end of a tube and an objective in the lower end of the tube. The magnifications for compound microscopes range from about forty times (40 X) to about 1,000 X.
- **Dissecting microscope**
Dissecting microscopes are essentially compound microscope limited to low magnification. Their magnification ranges from about five times (5x) to about sixty times (60x).
- **Electron Microscope**
Electron microscopes use a focused beam of electrons to produce an enlarged image that is typically projected on a fluorescent screen or photographic film.

Design of Compound Microscope

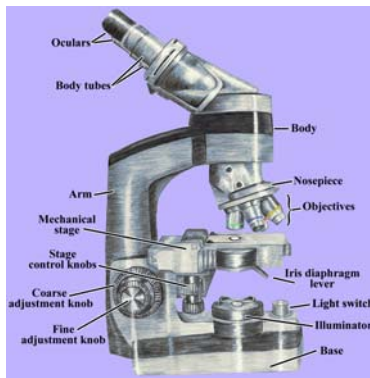


Figure 2.1

- **Base**
The base is the lower part of the microscope. The base allows the microscope to sit on a flat surface, it supports the upward extension called an arm, and it houses a light source.
- **Light Source**
An electrical light source (small bulb) with an associated power switch and electrical cord is found at the base of the microscope (some microscopes use a mirror instead of an electrical light source).
- **Arm**
The arm is the upward part that extends from the base. The arm curves to position the body of the microscope over the stage. The arm has focusing knobs near the base.

Focusing Knobs

- Focusing knobs extend from each side of the arm and are the external part of the focusing mechanism. Focusing knobs are named as to their function as either **coarse adjustment knobs** or **fine adjustment knobs**. Coarse adjustment knobs are always larger than the fine adjustment knobs. Usually, both the coarse and fine adjustment knobs are part of the same focusing mechanism (coaxial focus), with the larger coarse adjustment being the inner knob.



Figure 2.2 (coaxial focus)



Figure 2.3

Body of Microscope

The body of the microscope is found at the top of the arm and is positioned over the microscope's stage. Located on the lower surface of the microscope's body is the **nosepiece** with optical magnifiers called **objectives**. Associated with the top surface of the microscope's body are one or more **tubes** (body tubes) each with an optical magnifier called an **ocular**.

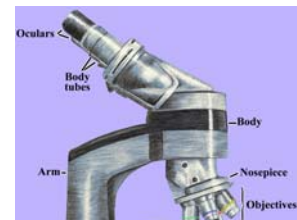


Figure 2.4

Ocular

The ocular (eyepiece) is the lens system that one looks into to make an observation. If the microscope has one ocular, the microscope is described as a monocular compound microscope. Binocular compound microscopes have two oculars. A third ocular may be added to the microscope (trinocular) and is used for photographic imaging while directly viewing the specimen.



Figure 2.5

Nosepiece and Objectives

- The nosepiece is located on the bottom surface of the microscope's body. It holds one or more optical magnifiers called objectives.
- If the microscope's nosepiece has two or more objectives the nosepiece rotates so that each objective can be selected for use. The rotating nosepiece has an alignment stop at each objective and the objective must "click" into the stop for perfect optical alignment.

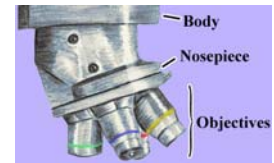


Figure 2.6

Objectives

- An optical objective is a lens system that is used for primary magnification of the specimen. Compound microscopes usually have two or more objectives attached to the rotating nosepiece. Microscopes are usually supplied with three or four objectives.

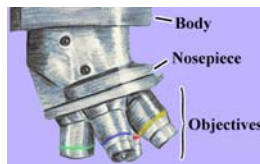


Figure 2.6

Name of Objectives

- The objectives that are usually supplied for a compound microscope are called **scanning power** (usually with a black band), **low power** (usually with a green band), **high power** (usually with an orange band), and **oil immersion** (usually with a red band).



Figure 2.7

Positioning of Objectives

- An objective is moved into position by rotating it until it is centered exactly over the center of the condenser lens seen at the center of the stage. When an objective is moved into position it must be "clicked" into its alignment stop.



Figure 2.8

Magnification of Objectives

Objectives are manufactured with a wide range of magnifications. Usually, the objectives are named, coded with a colored band, and have the following magnifications:

- scanning power = 3.5x or 4x - with a black band
- low power = 10x - with a green band
- high power = 40x or 43x - with an orange band
- oil immersion = 97x or 100x - with a red band



Figure 2.9

Working Distance and Focal Point of Objectives

The working distance is the distance between the objective and the microscope slide's specimen. The working distance will decrease with each objective of increasing magnification.

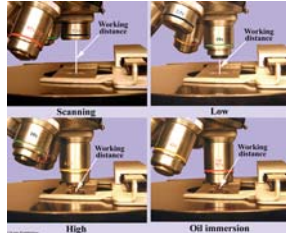


Figure 2.10

Depth of Field

The depth of field is the distance range within which an object (the specimen) looks sharp. The depth of field distance will decrease with each objective of increasing magnification.

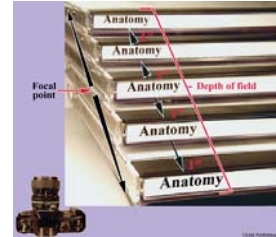


Figure 2.11

Scanning Objective

- The scanning objective has the deepest depth of field; it has the greatest distance of sharp focus on each side of the focal point. It is not possible to accurately determine the sequence of silk threads.

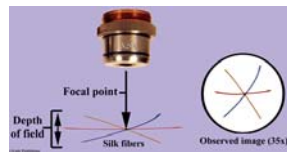


Figure 2.12

Shallow Depth of Field

- A lens with high magnification has a shallow depth of field. When a high magnification lens is focused on the center label, the depth of field extends less than one inch on each side of the focal point.

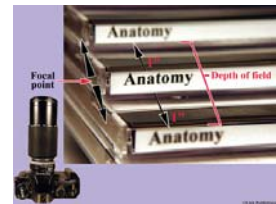


Figure 2.13

Shallow Depth of Field

- Illustrated depth of field when using the oil immersion objective and the microscope slide with three overlapping threads. Only one thread can be observed to be in focus at a time (depth of field is the same as the diameter of the thread).

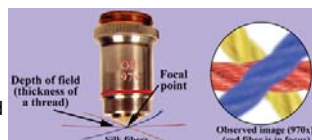


Figure 2.14

Field of View

The field of view (or field) is the area visible through the microscope. The field decreases with each objective's increasing magnification.

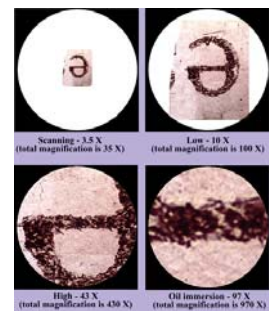


Figure 2.15

Total Magnification Power

The total magnification (the number of times your specimen is magnified) is determined by multiplying the magnification of the objective by the magnification of the ocular.



Stage

The stage is the platform that holds the specimen (usually fixed to a glass microscope slide) for examination. A variety of stages are available on microscopes.

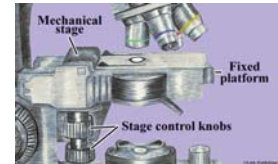


Figure 2.16

Stage

- Mechanical stage
- To avoid damage to microscope slides:
- do not allow the slide to slip under the clips
 - do not allow the clips to "snap" back onto the slide

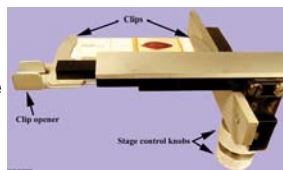


Figure 2.17

Condenser Lens

The condenser lens system focuses light from the illuminator to the specimen. Thus, the observed specimen is always centered above the condenser lens.



Figure 2.18

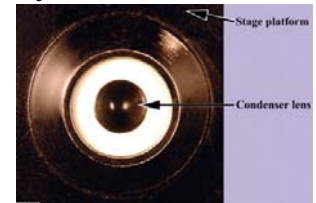


Figure 2.19

Condenser Lens

- Some microscopes have a condenser that is moved up and down by an adjustment knob. Generally, if the condenser is moveable, it is used in its uppermost position.

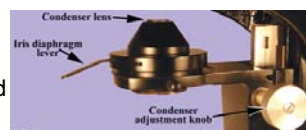


Figure 2.20

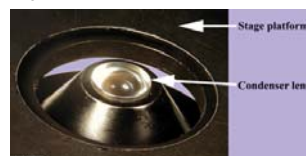


Figure 2.21

Diaphragm - Iris or Disk

- The diaphragm functions to regulate the amount of light that illuminates the specimen. Two varieties of diaphragms are available with compound microscopes, the iris and the disk diaphragms.
 - The iris diaphragm is adjusted so that the specimen has the best appearance as to the amount of light and observed detail (resolution).

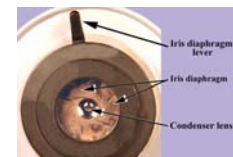


Figure 2.22

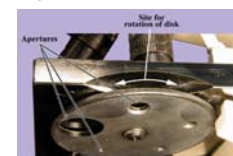


Figure 2.23

Care and Use of the Microscope

Setting-up the Microscope

- When you carry the microscope use **both of your hands**.
- Carefully sit the microscope on your table's surface **away from the edge**.
 - Lens paper** should be used to clean any optical surfaces. Never use paper towels, tissues, or other materials that are not specifically designed for lenses.
- Inspect your microscope.** Making sure that all of the components are present and in good condition. If a

Putting away the microscope

- Be sure that a microscope slide is **not** on the stage.
- Be sure that either the **scanning power** or low power objective is in position.
- If you used immersion oil, be sure that you have **cleaned (with lens paper) the oil** from your oil immersion objective.
- If the microscope has an electrical light source, unplug it by **pulling on the plug**.
- The **electrical cord** should be either gently wrapped around the base or carefully coiled upon itself.
- When you carry the microscope use **both** of your hands.
- Always return the microscope to its **proper cabinet**.
- Sit the microscope in its cabinet **carefully**.

Microscopic Specimens

Prepared microscope slides

- A common source for specimens is prepared microscope slides. Prepared microscope slides are expensive and should be used with great care by the microscopist.
 - Slides should **not be dropped** nor placed in precarious places such as tabletops and on books. Prepared slides should be cleaned with **lens paper** to avoid scratches.

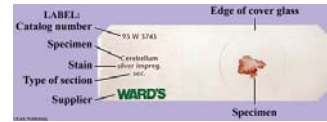


Figure 2.24

Wet Mount Slide Preparation

- A wet mount is a slide preparation that utilizes a liquid; usually water, for suspension of the specimen.
- The objective lenses should **NEVER** contact fluid or the cover glass.

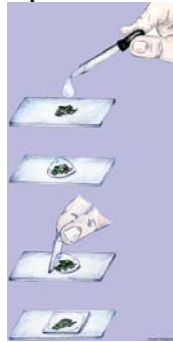


Figure 2.25

FOCUSING THE MICROSCOPE

- Before observing prepared microscope slides **read the label and visually observe your specimen**.
- Before placing the microscope slide on the stage make sure that either the **scanning objective** or the **low power objective** is in position on the nosepiece.
- The microscope slide must be moved (by the mechanical stage) so that the specimen is exactly **aligned at the center of the condenser lens**.
- Adjust the iris diaphragm** so that a middle level of light intensity is obtained.

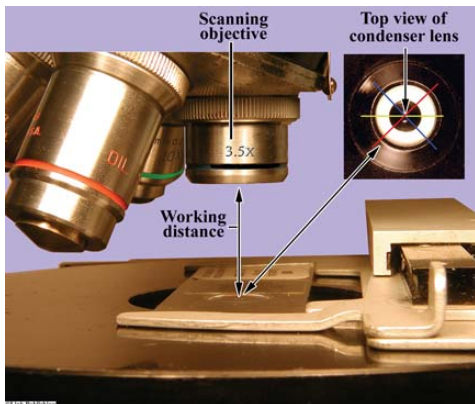


Figure 2.26

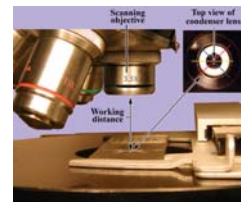


Figure 2.27

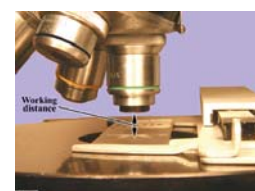


Figure 2.28



Figure 2.29

LAB ACTIVITIES

- **OBSERVATIONS**
Materials commonly used for introduction to microscopy and used in this study are:
- **Materials**
 - Prepared Microscope Slides
 - Letter "e" (a printed letter "e")
 - Silk fibers (colored threads that are crossed)
 - Exfoliated squamous epithelium (from mouth)
 - Millimeter ruler (flat clear-plastic metric ruler)
 - Blank slides and cover glasses
 - Toothpicks
 - Pond water (or cultured protozoa)

Observation of Millimeter Ruler Diameter of Field and Working Distance

- Position the metric ruler on the top of a blank microscope slide. Center the ruler in reference to the condenser lens. Begin observation with scanning objective.

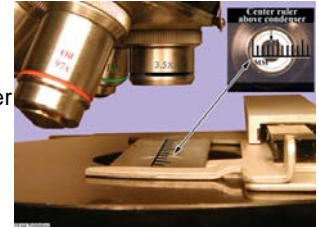


Figure 2.30

Diameter of Field

- Align one of the millimeter lines to the edge of the field, adjust the amount of light (iris diaphragm), obtain a sharp focus, and count the number of millimeters across the field (record in worksheet).

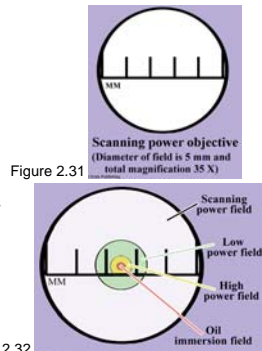


Figure 2.32

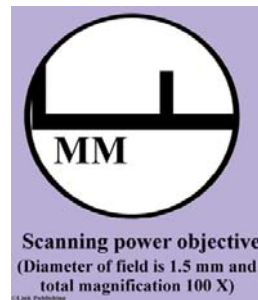


Figure 2.33



Figure 2.34

Observation of Letter "E" (printed letters)

- Visually observe the letter "e" on the microscope's stage and determine its physical orientation. (Is the letter "e" upside down, rotated upward, downward, etc.?) Look through the microscope and notice that the orientation of the letter "e" appears upside-down and backward.



Figure 2.35

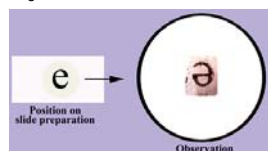


Figure 2.36

Diameter of the Field and Size of letter "e"

- If the field diameter is 5 mm, then each letter "e" is 1/5 of 5 mm or each letter is 1mm in width.

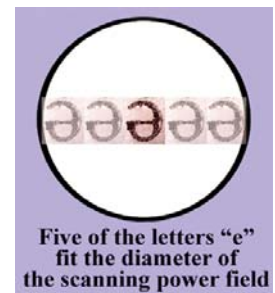
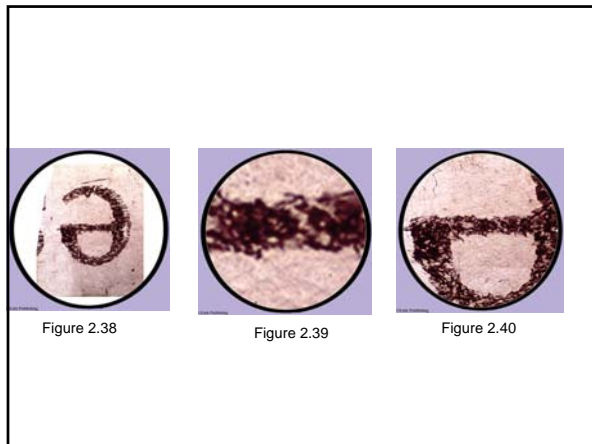


Figure 2.37



SILK FIBERS (Cross Colored Threads)

- Obtain a microscope slide preparation labeled "Silk Fibers" or "Cross Colored Threads." Typically, this preparation is of three different colored (red, blue, and yellow) silk threads that are positioned one upon another to cross at one point.

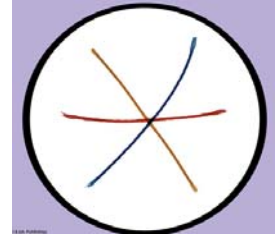
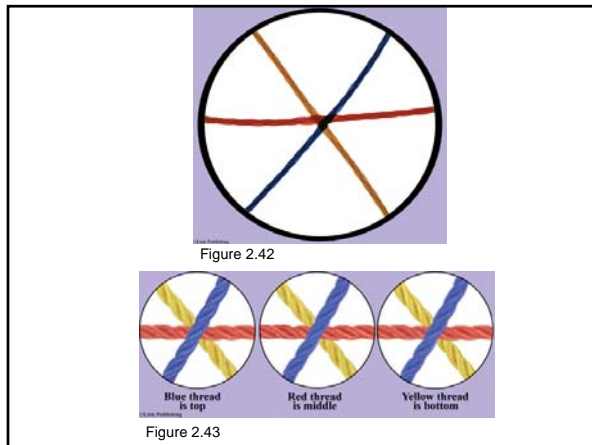


Figure 2.41

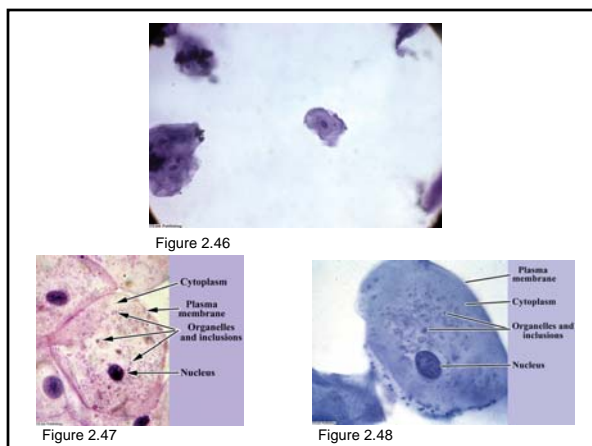


Exfoliated Squamous Cells

- The mouth is lined with a tissue covering called stratified squamous epithelium. Stratified squamous epithelium is described as multi-layered (stratified) flat (squamous) lining tissue (epithelium).



Figure 2.45



Observation of Pond Water or Protozoa Culture

- Observation of protozoa and other microscopic organisms gives additional opportunity in developing proficiency in microscopy and identification of cellular structures. Protozoa are mostly motile unicellular organisms. Paramecia and amoebas are common protozoa found in pond water.



Figure 2.49

Examples of organisms found in pond water.



Figure 2.50