Microscope Notes

- Microscope an instrument that produces an enlarged image of an object.
- Biologists use microscopes to study cells, cell parts, and organisms that are **too small** to be seen with the naked eye.
- Microscopes magnify and show details of the image.
- Two types of microscopes:
 - Light Microscope light passes through one or more lenses to produce an enlarged image of a specimen
 - Electron Microscope forms image of a specimen using a beam of electrons rather than light
- Some early microscopes:



- Zacharias Janssen Dutch spectacle maker who tested several lenses in a tube and discovered that nearby objects appeared significantly enlarged.
- Robert Hooke used a microscope to observe a **thin slice of cork**. The spaces he saw reminded him of the **small rooms** where monks lived, so he called them "**cells**," which he used to describe the **smallest**



units of life.

- He used microscopes with two and **three** lenses, but they didn't produce very detailed images.
- Drawings made by Robert Hooke:



- Anton van Leeuwenhoek Dutch merchant who learned how to grind lenses to make simple microscopes (have only one lens). These produced clearer and more enlarged images than Hooke's.
 - He is considered "The Father of Microscopy" and built over 500 different microscopes.
 - He was the first to **discover microorganisms** under a microscope by observing a **drop of pond water** filled with life. He called them "**tiny animalcules**."
 - He also saw and studied bacteria and the blood flow through capillaries in the tail of a fish.
 - Drawings made by Anton van Leeuwenhoek:



Parts of a Compound Light Microscope:

- 1. Body tube: Keeps the two sets of lenses a set distance apart.
- 2. **Rotating nosepiece**: Allows one to change between the objective lenses.
- 3-5. **Objective lens**: The second set of lenses in a compound microscope (usually 4x, 10x, 40x).
- 6. **Stage clips**: Hold the slide in place.
- 7. **Diaphragm**: Adjusts the amount of light that hits the slide from the light source.
- 8. Light source: Where light comes from to see image.
- 9. Ocular lens: The first lens in a compound microscope (usually 10x).
- 10. **Arm**
- 11. **Stage**: Where one puts the slide to view.
- 12. Coarse Adjustment Knob: Moves the stage up and down very quickly.
- 13. **Fine Adjustment Knob**: Moves the stage up and down very slowly.
- 14. Base



- Compound Light Microscope
 - Light passes through the specimen on the slide and uses two lenses to form its image.
 - Capable of two things (below) that vary in different microscopes:
 - **Magnification**: a measure of how much the image is **enlarged**

Total magnification = (ocular lens)(objective lens being used)

[The ocular lens usually has a 10x magnification, but that can vary.]

4x objective lens = (10x)(4x) = 40 times total magnification 10x objective lens = (10x)(10x) = 100 times total magnification 40x objective lens = (10x)(40x) = 400 times total magnification



• **Resolution**: a measure of the **clarity** of an image; how clear the details are



The Electron Microscope

• **Resolution** is the limiting factor to a light microscope since the **greater the magnification is**, **the less it is able to resolve the image**. At magnifications **beyond 2,000x**, the image becomes blurry, but **electron microscopes** can be used at greater magnifications.



- Characteristics of the Electron Microscope
 - A beam of electrons is used to produce an enlarged image of the specimen (it does not use light).
 - This electron beam and the specimen must be **placed inside a vacuum chamber** so that the electrons in the beam do not bounce off gas molecules in the air.
 - Since living things cannot survive in a vacuum, the electron microscope cannot be used to view living cells.

• Much more powerful than light microscopes.



- There are two types of electron microscopes:
 - 1. Transmission Electron Microscope (TEM)



- Uses a **beam of electrons** transmitted through a very **thinly sliced specimen**. **Magnets** guide the beam of electrons toward the specimen, and the image is produced to view.
- Magnification to **200,000 times**.
- 2. Scanning Electron Microscope (SEM)



- Provides detailed **3-D images**.
- The specimen is **sprayed with a fine metal coating** (it is **not sliced** to view as in the TEM).
- As the beam of electrons is passed over the specimen's surface, the **metal coating emits a shower of electrons**, and a 3-D image of the **specimen's surface** is produced to view.

Rules for Using the Compound Microscope

- 1. <u>ALWAYS</u> carry the microscope with **one hand holding the arm and your other hand supporting under the base**.
- 2. Plug in the cord and turn on the light source of the microscope.
- 3. Place your slide on the stage and arrange the stage clips to hold the slide in place.
 - Keep the stage dry and <u>ALWAYS</u> make sure that your slide is dry, especially the bottom, before putting it on the microscope.
- 4. Always start with the **4x objective lens (should already be on this from when the microscope was put away). Focus** this objective lens using the **coarse adjustment knob**.

- 5. Once the image is in focus, **carefully swing the 10x objective lens in place** and focus this objective lens using the **coarse adjustment knob**.
- 6. Once the image is in focus, VERY carefully swing the 40x objective lens into place BE SURE TO <u>NOT</u> TOUCH THE SLIDE. Focus this objective lens using <u>ONLY</u> the fine adjustment knob.
 NEVER use the coarse adjustment knob while using the high power objective lens (40x).
- 7. Make observations and take notes as needed before preparing to put the microscope away (#8-11).
- 8. Lower the stage using the coarse adjustment knob.
- 9. Swing the objective lens back to low power (4x).
- 10. Turn off light source, unplug and neatly wrap cord around microscope (<u>NOT</u> around lenses or light source).
- 11. Place protective cover over microscope before you put the microscope away.

Preparation of a Wet Mount Slide

- Wet mount slides are used to view living organisms, such as ones that need to be kept moist, and any liquid substances.
 - 1. Using the appropriate tool, put your specimen in the **center of a clean and dry slide**.
 - 2. Add one large solid drop of water over your specimen (water should not move on slide).
 - 3. Hold a clean and dry coverslip at a 45° angle over your specimen/water drop. Let one edge of the coverslip touch an edge of the water drop.
 - 4. <u>Gently</u> drop the rest of the coverslip into place want to avoid getting air bubbles (the whole slip should touch water). *Remember to keep the rest of the slide [and microscope stage] dry!*

How to Measure Under the Microscope

- The **micrometer** (μ m) is the unit of measurement used to measure things under the microscope. • One micrometer = 0.000001 meter (10⁻⁶).
- To estimate the size of each cell, use the diameter of each objective lens shown below.

The **10x objective lens** has a field of view with a **diameter of 1,500 μm**.



The size of the cell would be about 500 μ m.

The **40x objective lens** has a field of view with a **diameter of 375** μ m.



The size of the cell would be about 100 μ m.