

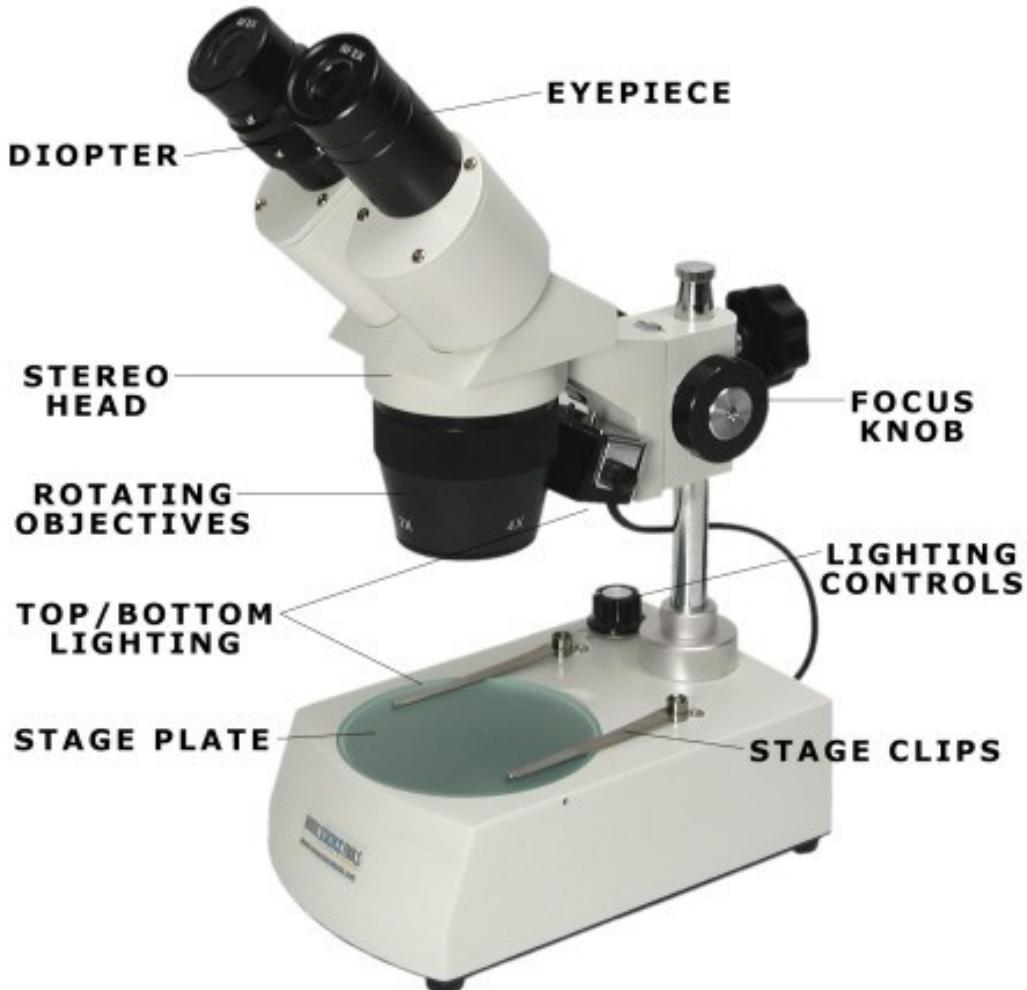
Early microscope

MICROSCOPES

- One of the most important inventions in the advancement of Biology

1. Simple Microscopes

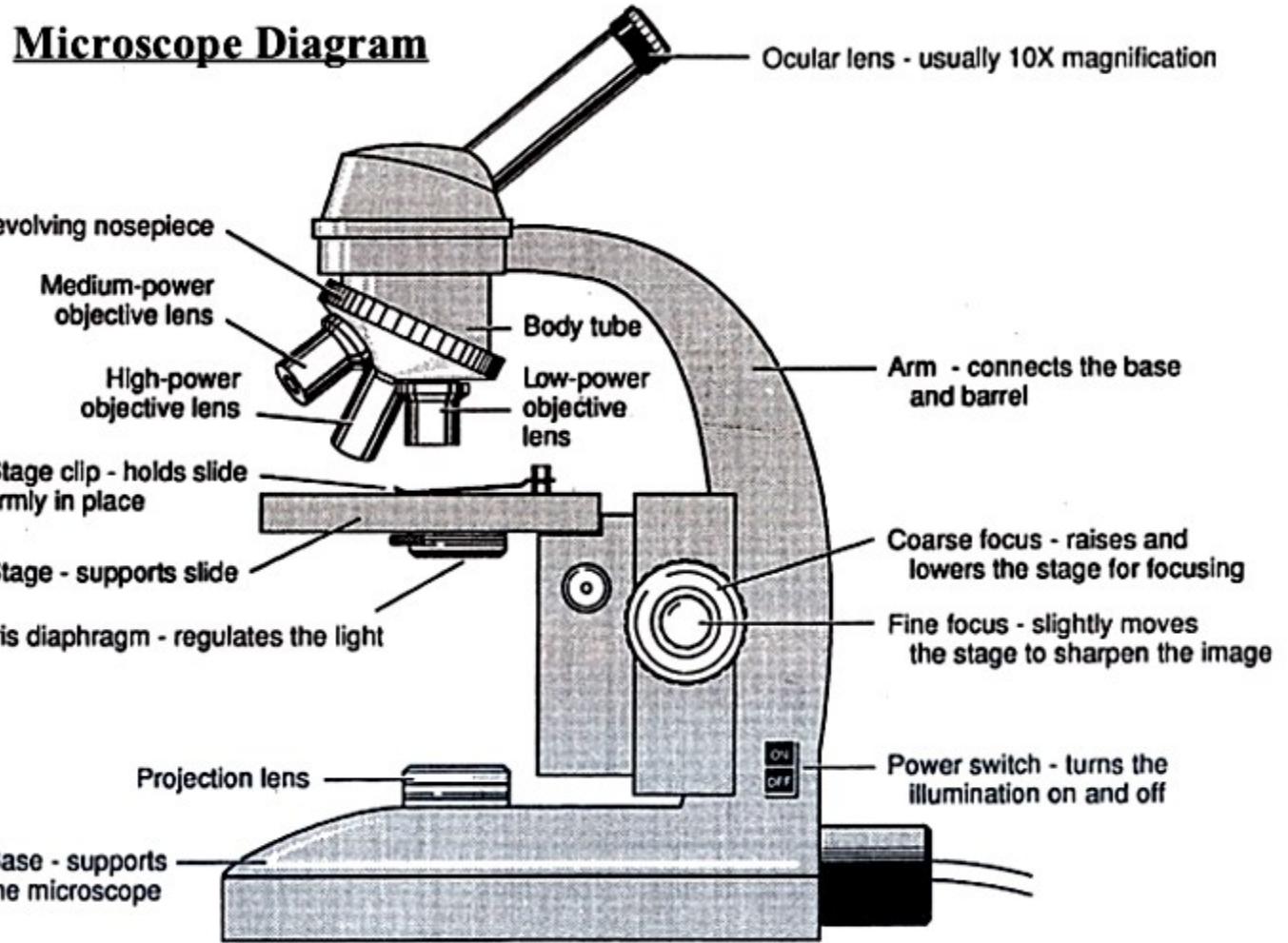
- ie. magnifying glass, stereoscope (dissecting scope)
- have a single lens or a pair of lenses combined
- uses **reflected light**
 - = light rays shone **on** an object are **refracted (spread)** as they pass through the convex lens(es) = enlarges object



- used for **low power magnification** of a sample
- image is **3 dimensional**

2. Compound Microscope

- has 2 separate lenses: **ocular** (monocular / binocular) & the **objective**
- uses **transmitted light**
 - = light passes **through** the object on the slide and is focused by the 2 lenses (mirrors help)



→ **Magnification** = how many times the specimen has been enlarged

Eyepiece x Objective = Total Magnification

$$10 \quad \times \quad 40 \quad = \quad 400x$$

→ **Resolution** = how clear the image is

: as magnification increases, clarity **decreases**

= **stains** and **reducing light intensity** are often used to make structures easier to see

→ **Field of View** = is the diameter of the circle of light

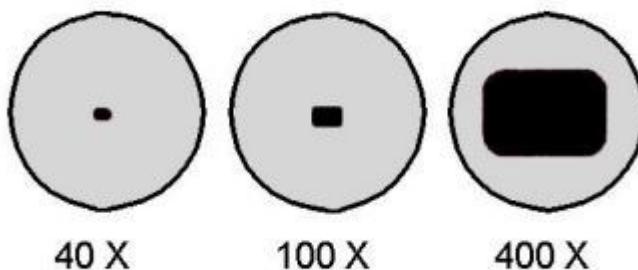
- varies depending on the **microscope** used & **objective lens** that is in place
- measured in μm (1 mm = 1000 μm)



Calculating the Field of View

When you are viewing an object under high power, it is sometimes not possible to determine the field of view directly using a ruler, therefore it must be calculated. Remember this:

THE HIGHER THE POWER OF MAGNIFICATION, THE SMALLER THE FIELD OF VIEW!!



The diameter of the field of view under high power can be calculated using the following equation:

$$\frac{\text{magnification on low power}}{\text{magnification on viewing power}} \times \text{diameter of low power field of view}$$

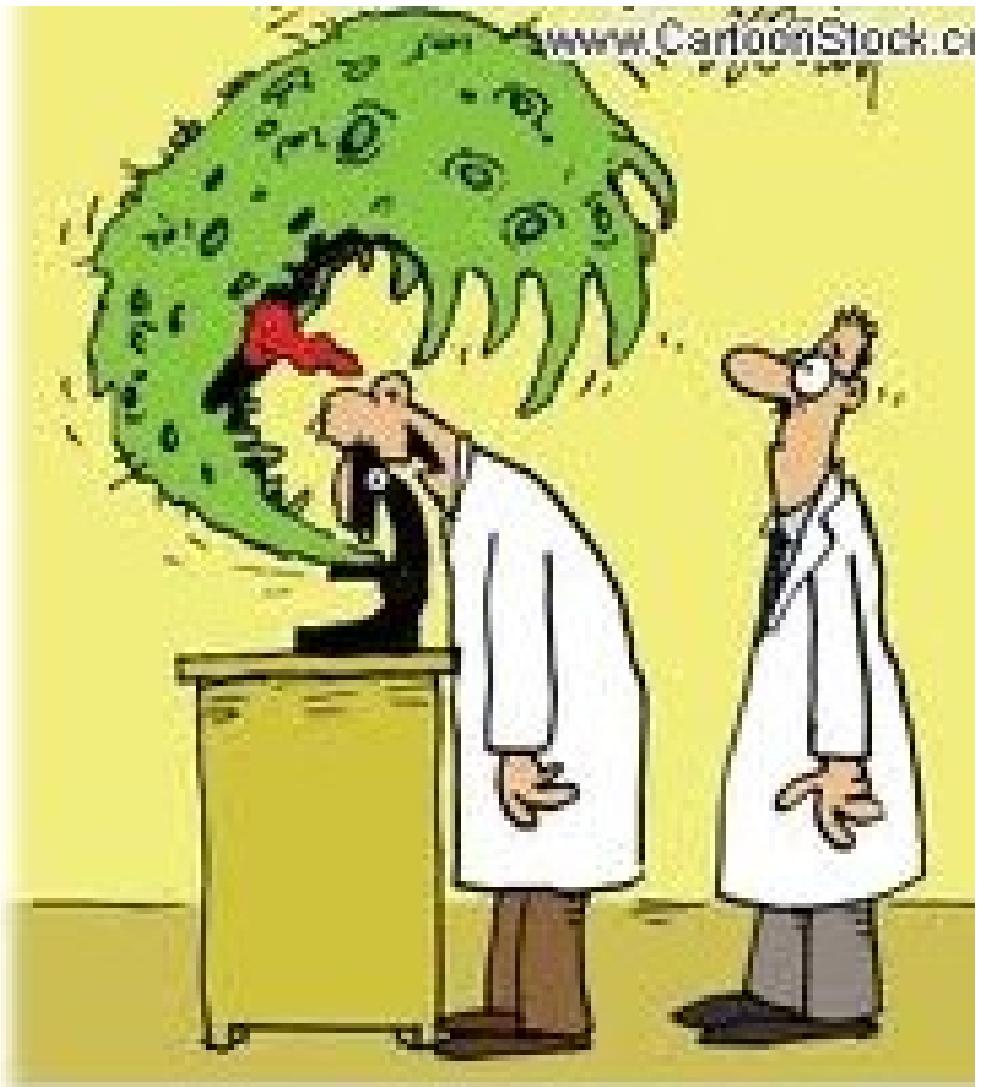
For example, if you determine that your field of view is 2.5 mm in diameter using a 10X ocular and 4X objective, you will be able to determine what the field of view will be with the high power objective by using the above formula. For this example, we will designate the high power objective as 40X.

$$\text{Convert mm to } \mu\text{m} \rightarrow 2.5\text{mm} = 2500\mu\text{m}$$

$$\frac{\text{Low Power Mag}}{\text{High Power Mag}} \times \text{Low Power F of V} \rightarrow \frac{4}{40} \times 2500\mu\text{m} = 250\mu\text{m}$$

If the medium power objective on this same microscope magnifies 10x, what would be the diameter of the field of view?

$$\frac{\text{Low Power Mag}}{\text{Medium Power Mag}} \times \text{Low Power F of V} \rightarrow \frac{4}{10} \times 2500\mu\text{m} = 1000\mu\text{m}$$



"I DON'T KNOW WHAT THIS IS, BUT YOU
SHOULD SEE HOW FAST IT'S GROWING

Estimating the Size of the Specimen Under The Microscope

Objects observed with microscopes are often too small to be measured conveniently with a ruler in millimeters. By comparing the size of a specimen to the diameter of the field of view, the actual size of the specimen can be calculated.

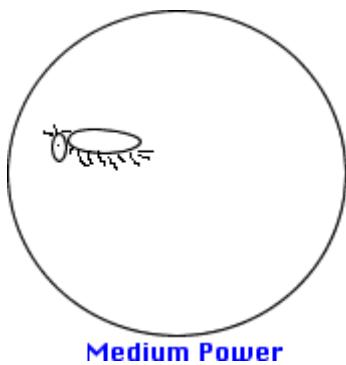
To estimate the size of an object seen with a microscope:

- a) position the specimen against one edge of the FOV
- b) estimate the specimen that fit across the diameter of your field of view using the **longest side of the specimen**
- c) calculate specimen size using the formula:

$$\text{Size of Specimen} = \frac{\text{diameter of the field of view}}{\# \text{ of specimens that fit across}}$$

Examples:

- a) Calculate the size of the following specimen if the diameter of the field of view is $3000\mu\text{m}$.



$$\text{Size of Specimen} = \frac{\text{diameter of the field of view}}{\# \text{ of specimens that fit across}}$$

(guesstimate 3)

$$= \frac{3000\mu\text{m}}{3}$$
$$= 1000\mu\text{m}$$

*** As you are "guessing" how many fit across an acceptable size range is determined. Anything between $860 - 1200\mu\text{m}$ is acceptable

- b) If the field of view above measured 5mm instead of $3000\mu\text{m}$, calculate the size of the specimen.

$$\text{Size of Specimen} = \frac{\text{diameter of the field of view}}{\# \text{ of specimens that fit across}}$$
$$= \frac{5000\mu\text{m}}{3}$$
$$= 1667\mu\text{m}$$

(range = $1425 - 2000\mu\text{m}$)

Care and Use of Microscopes

1. Always use both hands to carry the microscope; one on the arm, the other supporting the base.
2. Use only lens paper to clean the lenses.
3. Coarse focus knob is ONLY used when the Low (Scanning) power objective is in place.
4. Do not remove any parts .
5. When finished, make sure the Low (Scanning) power objective is in place and the stage is completely lowered.
6. Wrap the cord around the arm and replace dust cover.

Trouble Shooting

Occasionally you may have trouble with working your microscope. Here are some common problems and solutions.

1. Image is too dark!

Adjust the diaphragm, make sure your light is on.

2. There's a spot in my viewing field, even when I move the slide the spot stays in the same place!

Your lens is dirty. Use lens paper, and only lens paper to carefully clean the objective and ocular lens. The ocular lens can be removed to clean the inside. The spot is probably a speck of dust.

3. I can't see anything under high power!

Remember the steps, if you can't focus under scanning and then low power, you won't be able to focus anything under high power. Start at scanning and walk through the steps again.

4. Only half of my viewing field is lit.

You probably don't have your objective fully clicked into place.

Guidelines for Microscope Drawings

1. Always use pencil and draw on 8 x 11 white paper portrait direction.
2. List Name, Date, Class, Group members in upper **right hand corner**.
3. Drawings must be **large** (no more than 2 per page), and single sided.
4. Drawing should be **centered** or slightly to the left.
5. Uses smooth, firm lines - **no sketching or shading**.
6. Use **parallel** lines drawn with a **ruler** to label.
7. Label on the **right side** only; line up labels **vertically**.
8. **Print** labels in lower case letters.
9. **Print** a title that is precise and descriptive **above** the drawing.
10. **Calculate** the size of your specimen below your diagram.

To calculate the size of the specimen

$$\text{Size of Specimen} = \frac{\text{Diameter of the field of view}}{\# \text{ of Times the Specimen fits Across}}$$

BE SURE YOUR FINAL ANSWER IS IN μm

