Microbiology

Microscopes

Microscope Introduction

- To magnify small things using lenses
- There are many different types of microscopes, with different magnifications and different uses
- Types of microscopes:
  - Simple Microscope* (10x)
  - Dissecting Microscope* (up to 300x)
  - Compound Microscope* (40-1000x)
  - Confocal Microscope
  - Electron Microscope (10,000,000x +)

Simple Microscope

- Single lens
- Light microscope
- $

Dissecting Microscope (FYI)

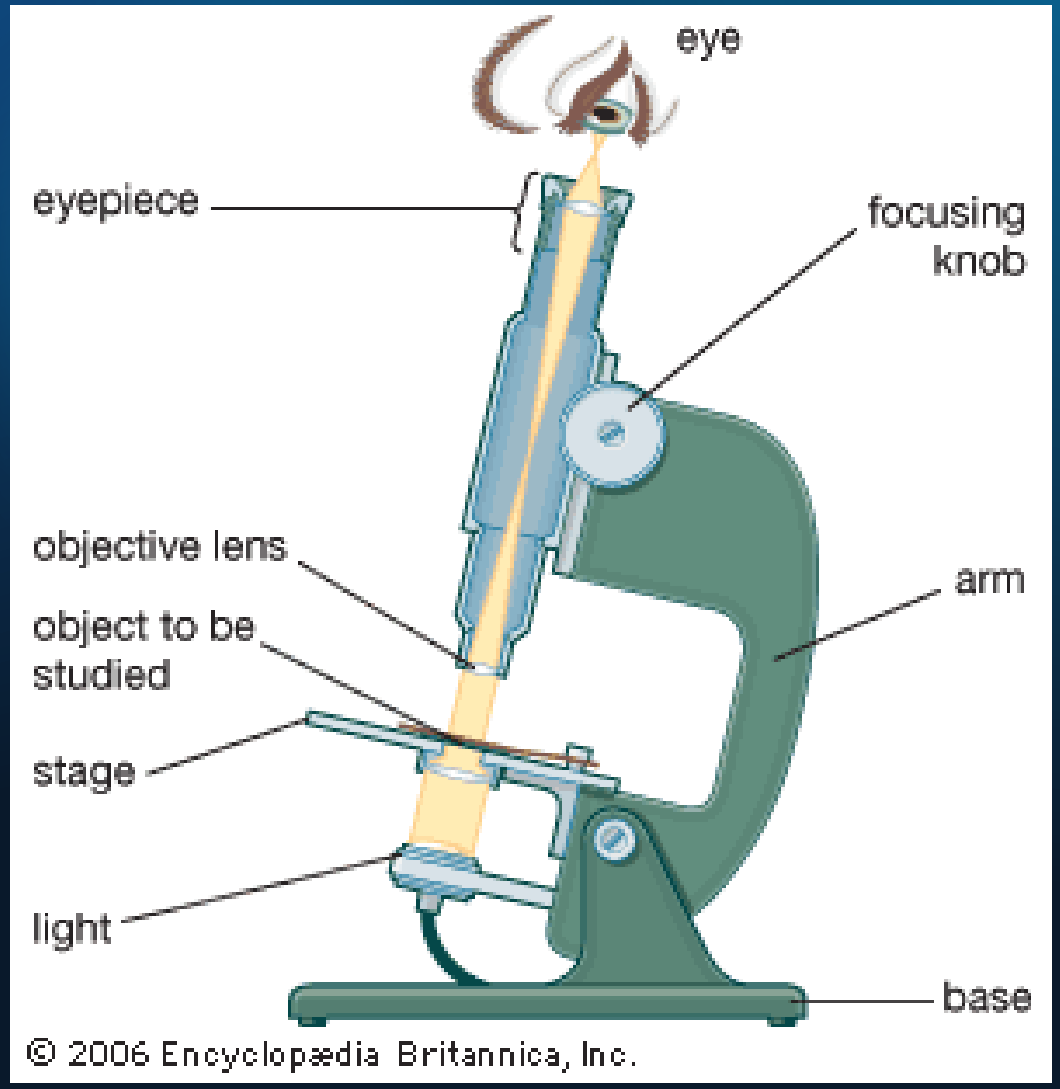
- Two lenses (10x ocular lens; 1x/2x/3x objective lenses)
- Light microscope for 3D objects, especially those that cannot be sliced thinly (e.g. living animals)

Compound Microscope

- Two lenses (10x ocular lens; 4x/10x/40x objective lenses)
- Light microscope for magnifying 2D slices (or very thin objects)
- $$

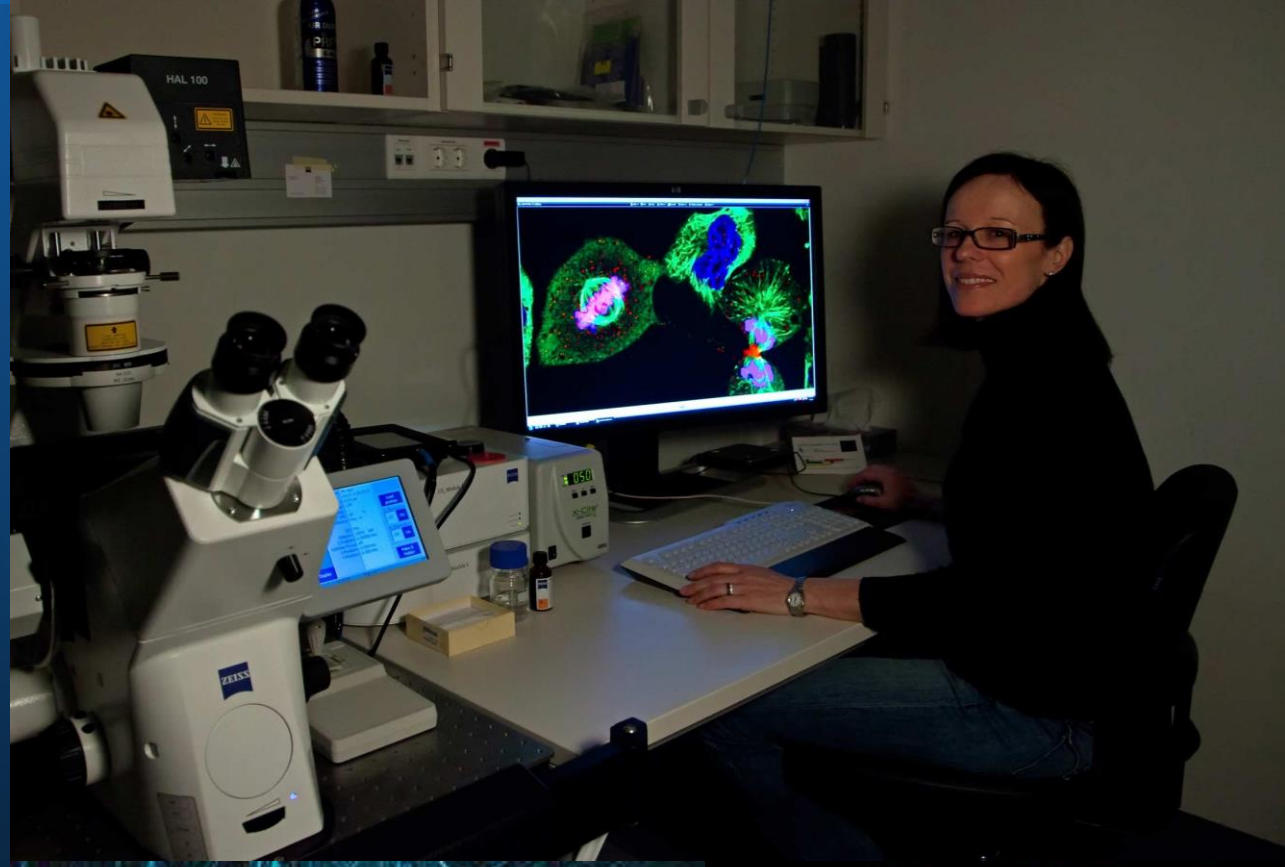
Compound Microscope

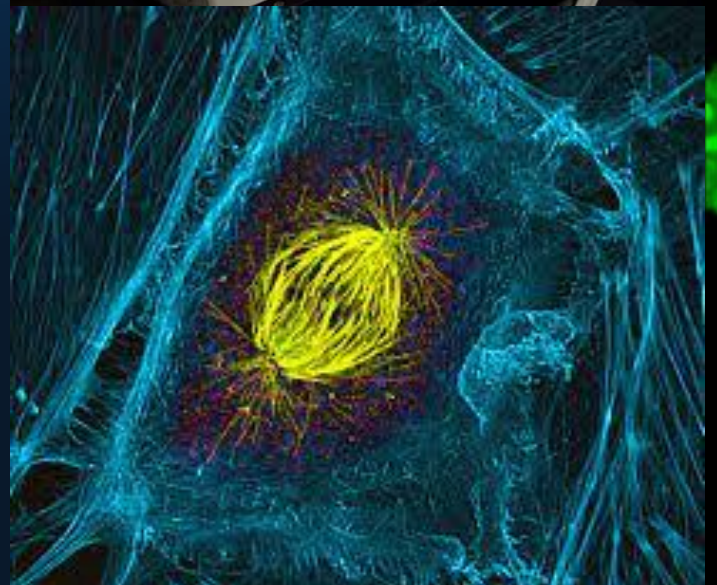
- Light travels up through the stage, through your specimen, through the objective lens, through the eyepiece (ocular lens) into your eye.
- If light's path is blocked or your object is not in focus, you will not see your specimen.

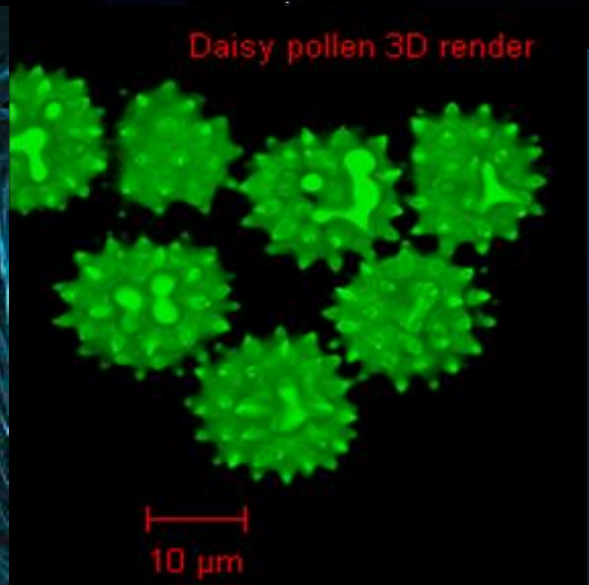


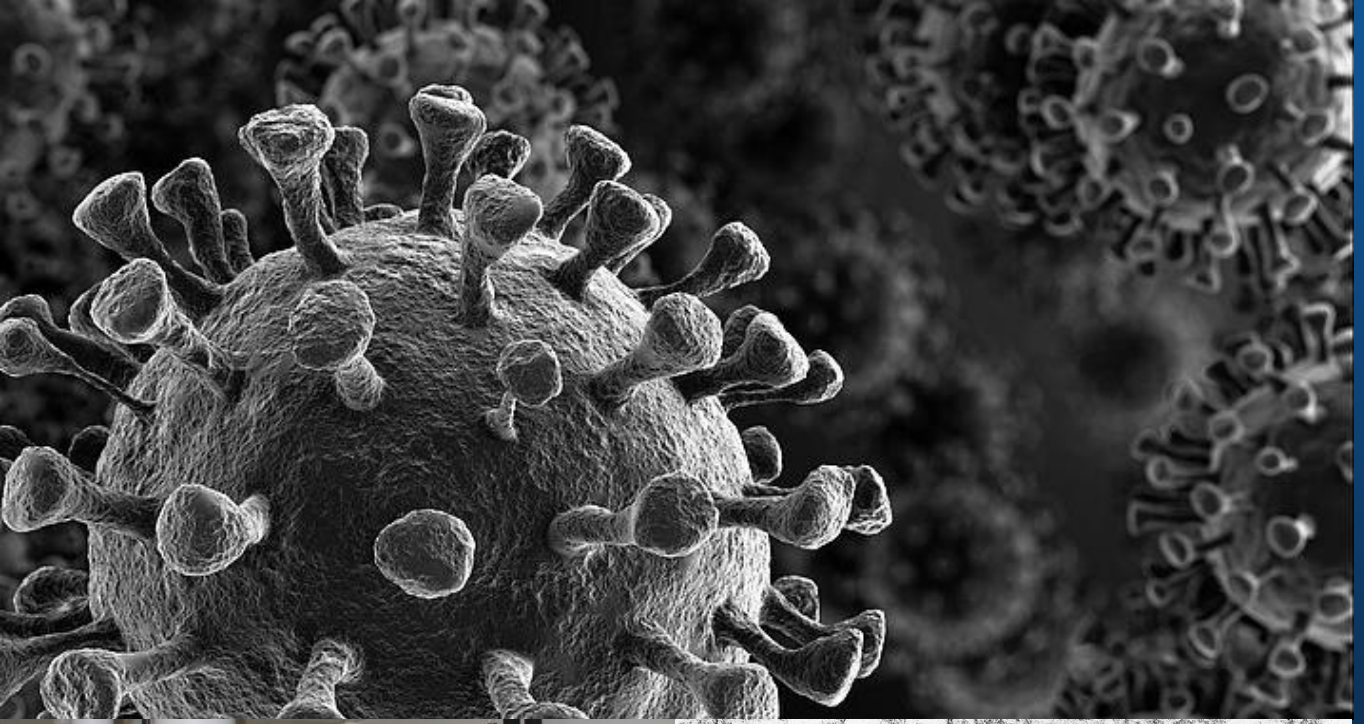
Confocal Microscope

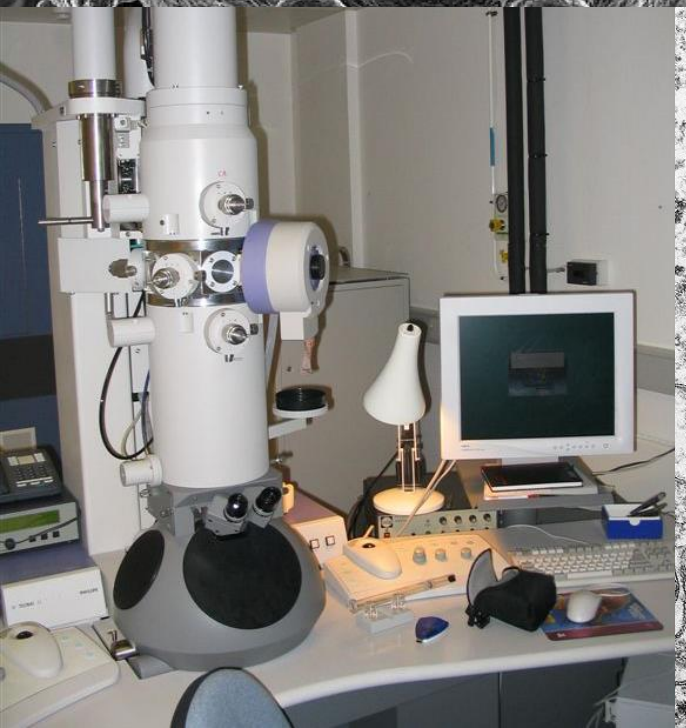
- Laser microscope
- Versatile: can take 2D images of slices or construct 3D model
- Sample preparation requires attaching fluorescent antibodies to proteins of interest
- $$$$$

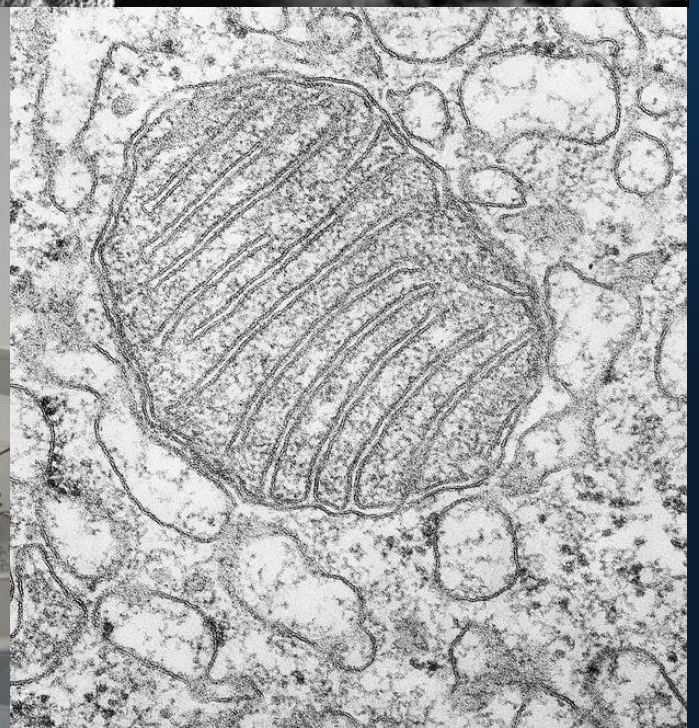












Electron Microscope

- Uses beams of electrons instead of light
- Magnifications of over 10,000,000x achievable
- Versatile: can image surfaces or very thin (50 nm!) slices
- $$$$$$$$$$$$

Preparing to Use Your Microscope

1. Double-check that it was put away correctly (low power).
2. Plug it in. Turn on the light.
3. Secure slide to stage, ensuring that specimen is illuminated.
4. Fix the focus:
   - Use the coarse adjustment knob to raise the stage all the way.
   - Turn the fine adjustment knob as far as it will go, then half-way back.

Focusing the Microscope

5. Look at the specimen with your naked eye. What colour and shape is it? Approximately how large is it?
   (This will help you avoid focusing on the wrong object.)
6. Double-check that your microscope is on low power.
7. Put your hand on the coarse adjustment knob and look through the eyepiece. Focus on the specimen with the coarse. (***Can't see it? Ask for help.***)
8. Center the specimen and adjust the diaphragm if necessary.

Focusing the Microscope

9. Switch to medium power. (***Do not go 'through' high power.***) You should see an enlarged, blurry version of the specimen.
10. Turn the coarse adjustment knob ***slightly*** to focus on the specimen.
11. Switch to high power, watching to ensure that the lens does not hit anything. You should see an enlarged, blurry version of the specimen.
12. Turn the ***fine adjustment knob*** to focus on the specimen.

Extremely Important

You will never see a specimen on a higher magnification if you can't see it on the lower one.

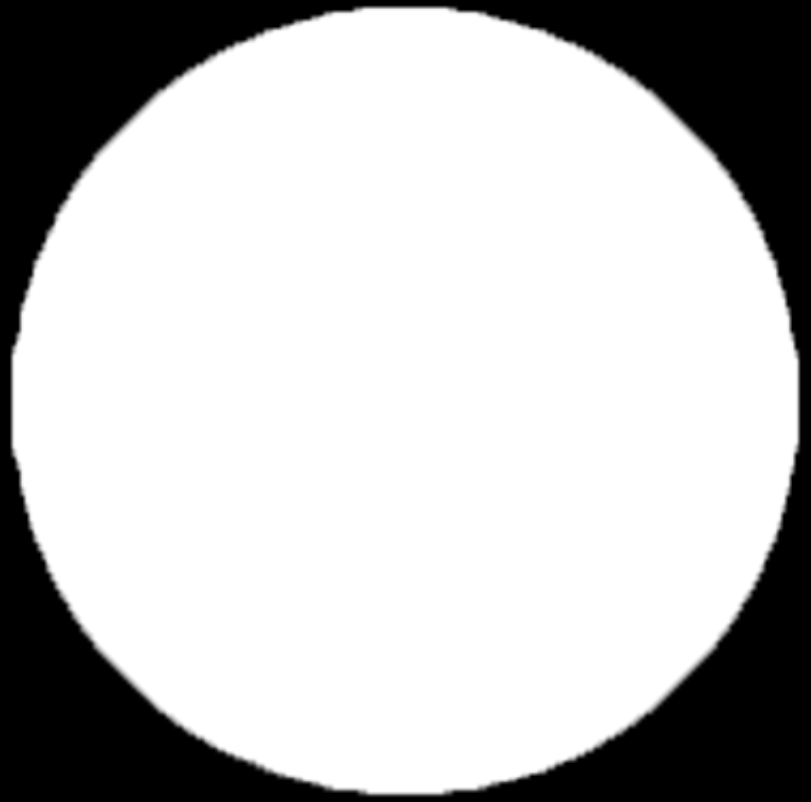
Never use coarse adjustment knob on high magnification.

- If you can't see the specimen on high, go back to medium.
- If you can't see the specimen on medium, go back to low.
- If you can't see the specimen on low, redo all the "Preparing to Use Your Microscope" steps and try to find the specimen again.
- If you still can't see the specimen, ask for help.

Troubleshooting #1

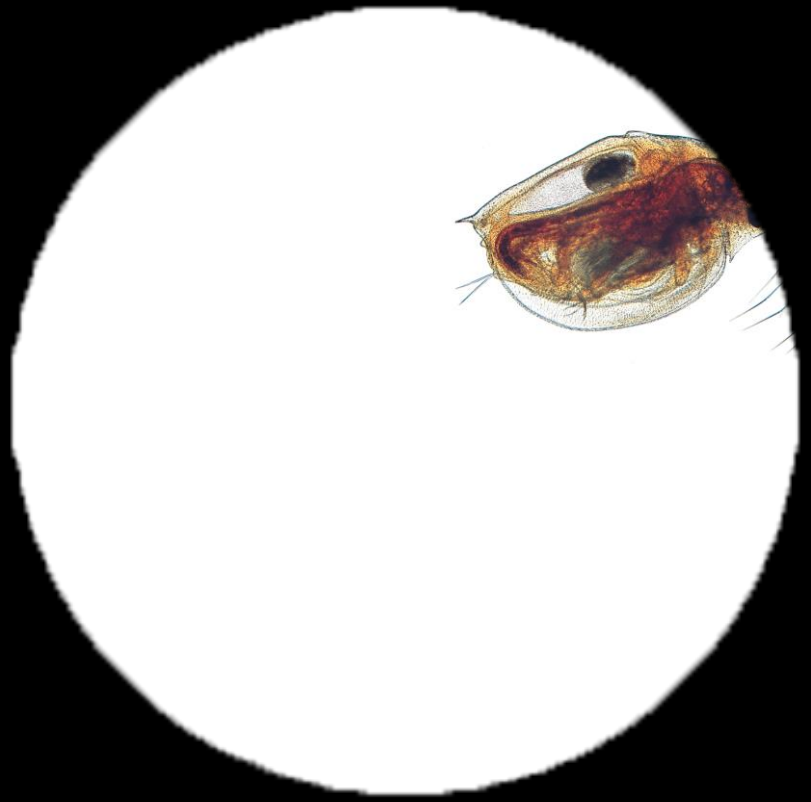
Low power: 40x total magnification

Solution:
Did you set up microscope correctly?
(Did you raise the stage all the way?)
Is specimen centered over circle?)



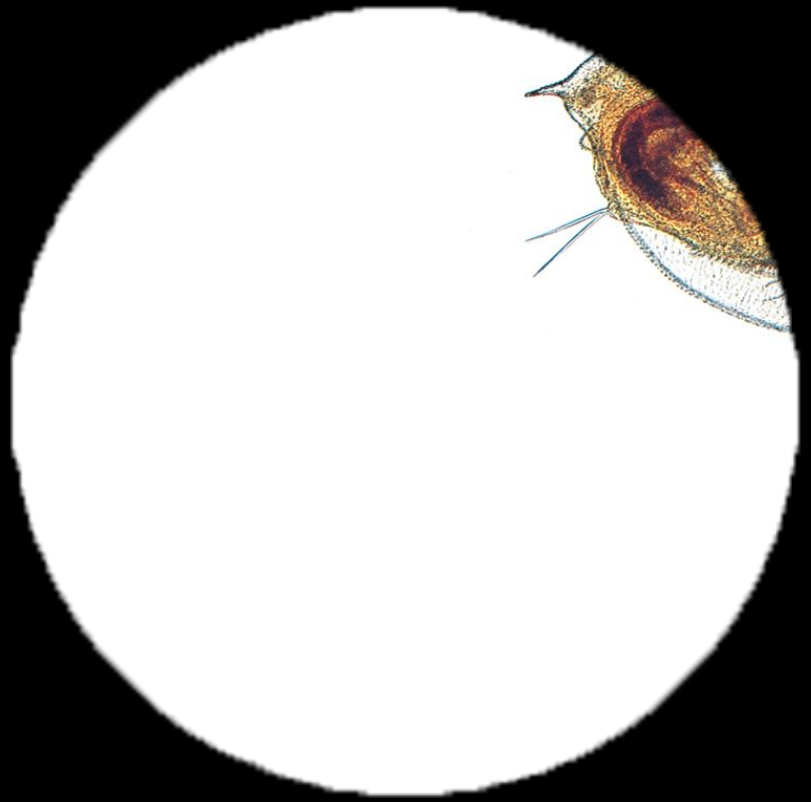
Troubleshooting #2

Low power: 40x total magnification



Troubleshooting #2

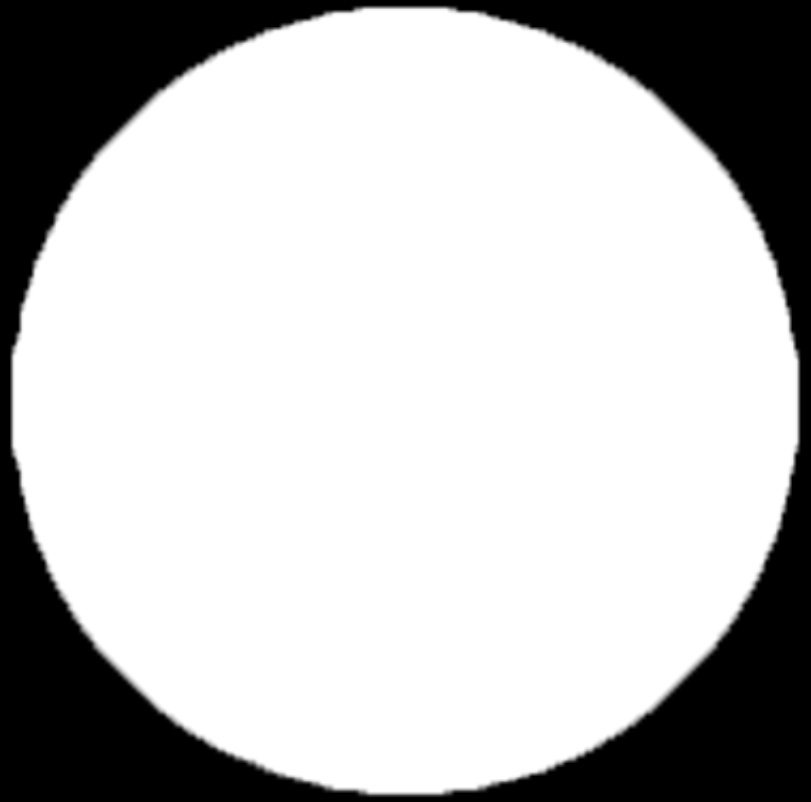
Medium power: 100x magnification



Troubleshooting #2

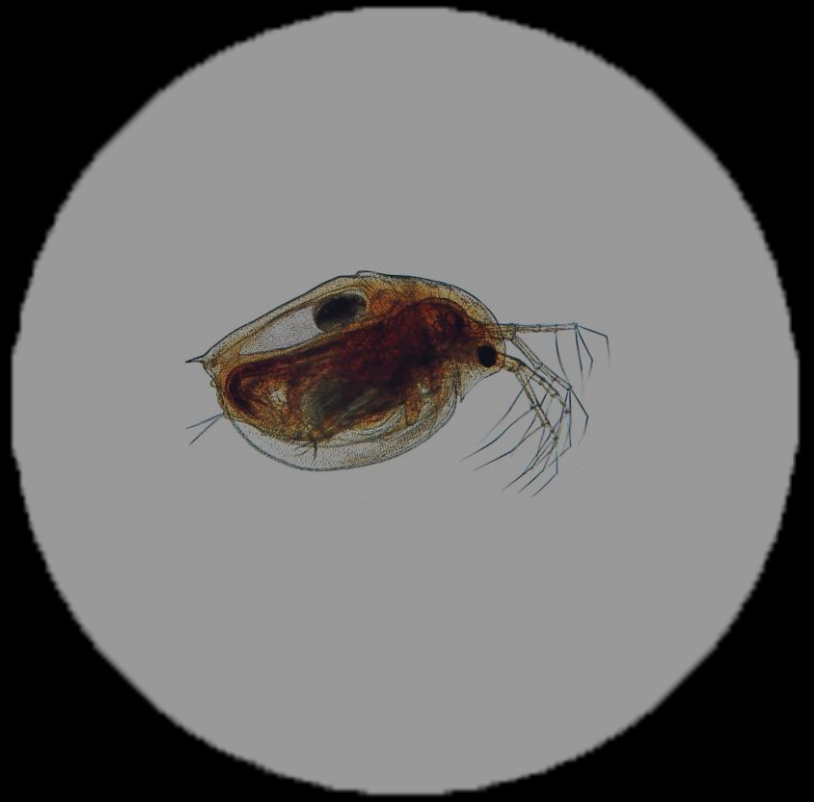
High power: 400x total magnification

Solution:
Center specimen before moving to higher magnifications.



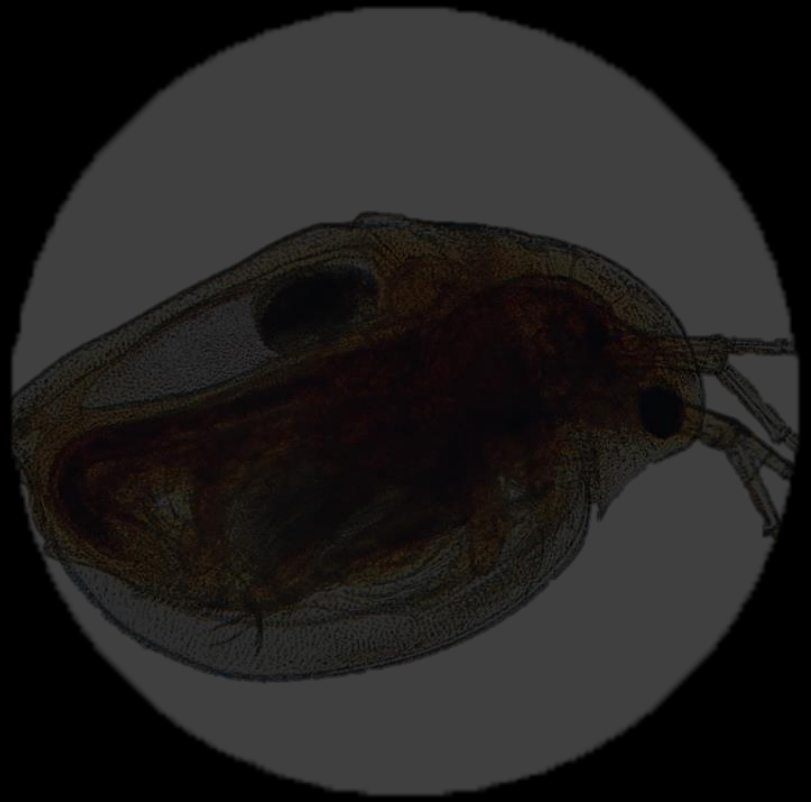
Troubleshooting #3

Low power: 40x total magnification



Troubleshooting #3

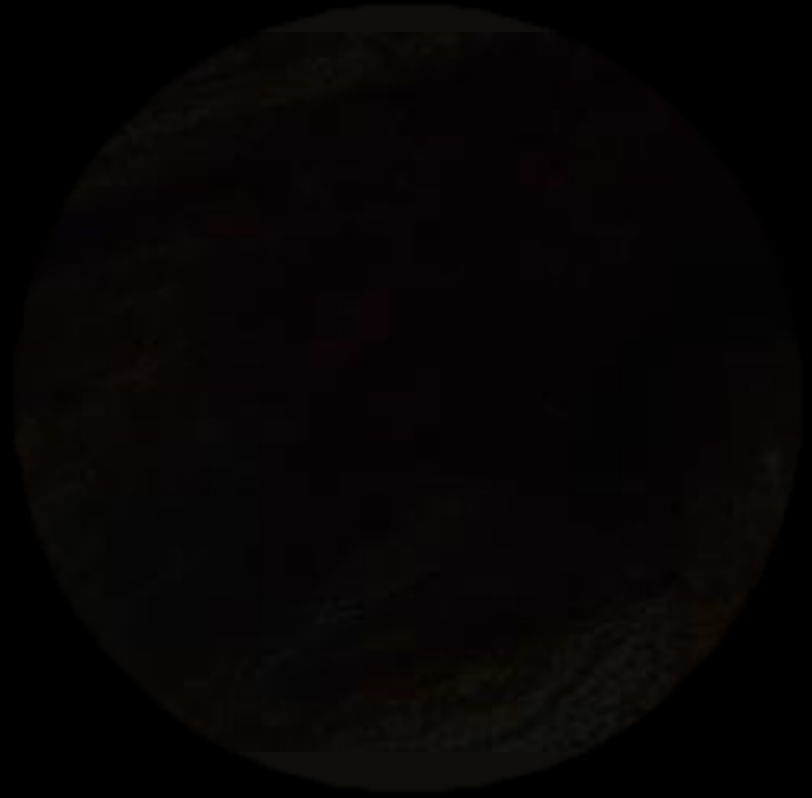
Medium power: 100x magnification



Troubleshooting #3

High power: 400x total magnification

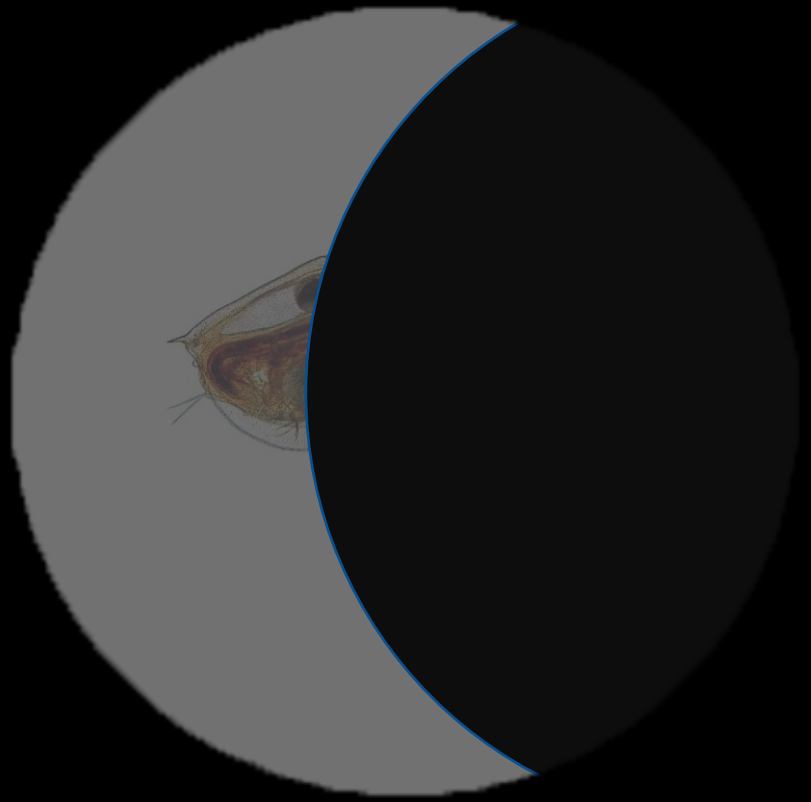
Solution:
Adjust the diaphragm to increase the amount of light.



Troubleshooting #4

Low power: 40x total magnification

Solution:
Ensure the diaphragm and objective lens are properly clicked into place.



Tips

Don’t forget about the real world.

- The microscope is a magnifying tool.
- If your eyes tell you the specimen is pink, and you’re focusing on a black squiggle, then that’s probably not what you’re supposed to be looking at!
- When switching objective lenses, always look at the lens to make sure it doesn’t bump anything.

Tips

Ask for help.

If you're not 100% sure about something, ask a neighbour or your teacher. If your teacher isn't available, go back through the steps and try to figure things out.

My eyes hurt.

Adjust the diaphragm to change the brightness. You can also take photos through the microscope, then look at your phone instead.

Tips

Eyepiece pointer.

Find something really cool? Some eyepieces are outfitted with pointers that you can use to point things out to other people.

“Help! I can’t see anything!”

Tips

High Power Objective Lens

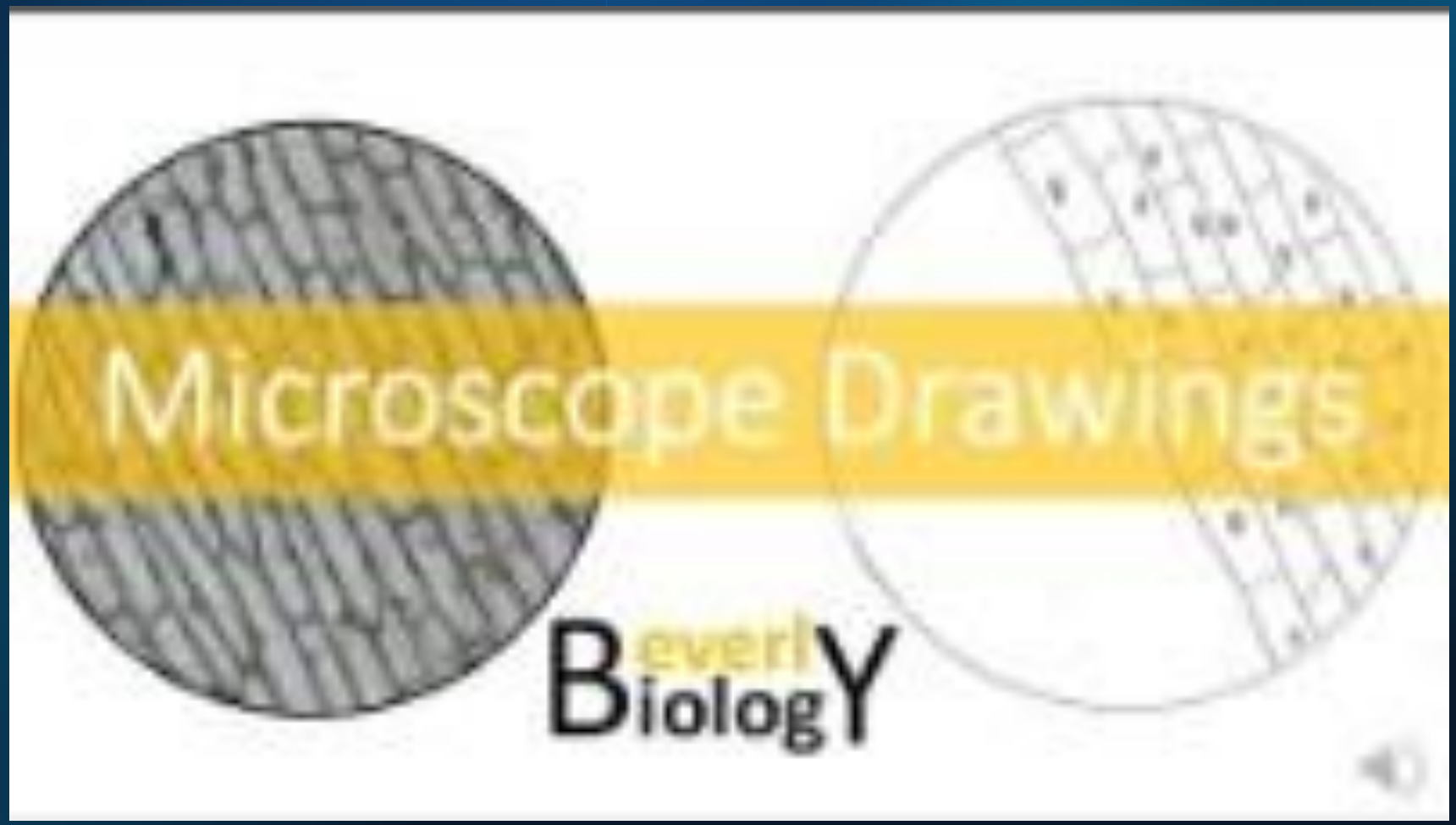
When you switch to the high power lens, it should already be very close to the slide. If not, go back to low power.

Never use coarse adjustment with high power lens.

Cleaning Up

- Set objective lens to low; wipe all objective lenses and the eyepiece with a special wipe.
- Remove slide from stage; return to teacher.
- Turn off and unplug the microscope.
- Wrap the cord around the microscope.
- Lower the stage all the way using the coarse adjustment knob.
- Rotate the eyepiece until it is directly above the arm.
- Return the microscope to where you got it from, holding the arm and the base.

Microscope Drawings



https://www.youtube.com/watch?v=NLKZ95aHqE4&ab_channel=BeverlyBiology

Microscope Drawings

1. Title:
   - Explains what the specimen is
   - Specific
2. Total magnification:
   - Power of the microscope
   - Multiply eyepiece magnification with objective magnification

Microscope Drawings

3. Accurate Drawing:
   - Pencil
   - No colour
   - Draw what you actually see
   - Don’t draw the pointer
   - If field of view is repetitive, just draw a representative portion of it
   - To scale (How much of the circle does it take up? Same specimen should appear more zoomed in at higher magnifications)

Microscope Drawings

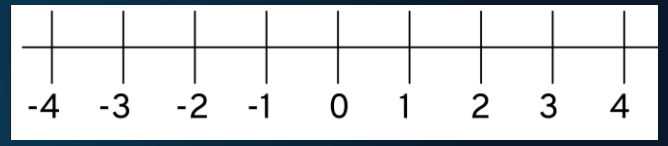
4. Labels
   - Straight line touching the object being identified
   - Do not use arrow
   - Written label outside field of view
   - Lines are horizontal and do not criss-cross

Unit Conversion Tutorial

1. Determine a conversion factor between your units.
   (Compare exponents → how many powers of ten apart are they?)

Multiplication Factor	Prefix	Symbol
$1,000,000,000 = 10^{9}$	giga	G
$1,000,000 = 10^{6}$	mega	M
$1,000 = 10^{3}$	kilo	k
$100 = 10^{2}$	hecto	h
$1 = 1 = 10^{0}$		
$0.01 = 10^{-2}$	centi	c
$0.001 = 10^{-3}$	milli	m
$0.000001 = 10^{-6}$	micro	μ
$0.000000001 = 10^{-9}$	nano	n

Memorize!



Example: mm (10^{-3}) and cm (10^{-2})
cm is 10x bigger
1 cm = 10 mm

Example: um (10^{-6}) and nm (10^{-9})
um is 10^{3} or 1,000x bigger
1 um = 1,000 nm

Example: km (10^{3}) and cm (10^{-2})
km is 10^{5} or 100,000x bigger
1 km = 100,000 cm

Unit Conversion Tutorial

1. Determine a conversion factor between your units.
2. Write your conversion factor as a fraction, with the desired unit on top and old unit on bottom.
3. Multiply by the top part of the fraction and divide by the bottom part of the fraction. Express your answer with the new unit.

Unit Conversion Tutorial

Example 1: convert 79 mm to cm.

1. 10 mm = 1 cm
2. $\frac{1\ cm}{10\ mm}$
3. $79\ mm \times \left(\frac{1\ cm}{10\ mm}\right) = 7.9\ cm$

Unit Conversion Tutorial

Example 2: convert 540 nm to um.

1. 1000 nm = 1 um
2. $\frac{1\ \mu m}{1000\ nm}$
3. $540\ nm \times \left(\frac{1\ \mu m}{1000\ nm}\right) = 0.54\ \mu m$

Unit Conversion Practise

Question	Answer
22 cm to mm	
1580 cm to m	
58,960,000 nm to mm	
0.0065 µm to nm	
28 µm to mm	
0.00003 m to nm	
600 nm to µm	
22,451 mm to µm	
890 mm to m	

Hint: Create your own practise questions (pick starting and final units), then google can help you check your answer!

Unit Conversion Practise

Question	Answer
22 cm to mm	220 mm
1580 cm to m	15.8 m
58,960,000 nm to mm	58.96 m
0.0065 µm to nm	6.5 nm
28 µm to mm	0.028 mm
0.00003 m to nm	30,000 nm
600 nm to µm	0.6 µm
22,451 mm to µm	22,451,000 µm
890 mm to m	0.89 m

Hint: Create your own practise questions (pick starting and final units), then google can help you check your answer!

Total Magnification

- Default magnifications (memorize!):
  - Eyepiece = 10x
  - Low objective = 4x
  - Medium objective = 10x
  - High objective = 40x
- Compound microscopes have two lenses working together.
- To calculate the **total magnification** of a compound microscope, you must ***multiply*** the magnifications of the two lenses.

Total Magnification

Example 1: *Calculate the total magnification of a compound microscope with a 15x eyepiece and a 40x objective lens.*

Total magnification = 15 x 40 = 600x

Example 2: *Calculate the total magnification of a compound microscope on medium power.*

Total magnification = 10 x 10 = 100x

Example 3: *Calculate the total magnification of a compound microscope on high power.*

Total magnification = 10 x 40 = 400x

Eyepiece

Every eyepiece has two values:

Magnification (of eyepiece lens only)

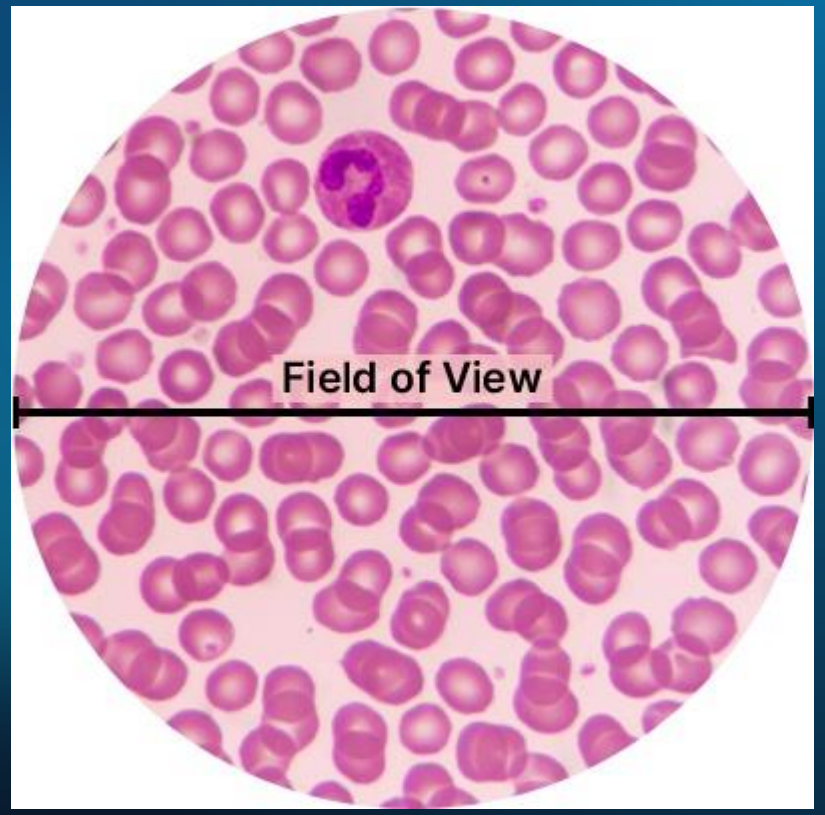
Field number:

- The *actual* diameter of the circle being viewed
- In millimeters (mm)

This eyepiece has a magnification of 10x and a field number of 20 mm.

Field of View

Field of View: diameter of observable area when looking through a compound microscope



$$field\ of\ view = \frac{field\ number}{objective\ magnification}$$

Field of View

Example 1: *Calculate the field of view on high power objective.*

$$field\ of\ view = \frac{20\ mm}{40} = 0.5mm$$

Example 2: *Calculate the field of view on low power objective.*

$$field\ of\ view = \frac{16\ mm}{4} = 4\ mm$$

Field of View

Example 3: *Calculate the field of view using an oil immersion lens (100x magnification). Convert your answer to micrometers.*

$$field\ of\ view = \frac{12\ mm}{100} = (0.12\ mm)\left(\frac{1000\ \mu m}{1mm}\right) = 120\ \mu m$$

Summary So Far

- Converting between metric units
- Calculating total magnification of a compound microscope
- Calculating the field of view after magnification

Up next: calculating specimen size.

Only one extra step: estimate how much of the field of view the specimen takes up.

Specimen Size: Daphnia

This is a water flea (*Daphnia magna*) on low power. If the eyepiece field number is 16mm, determine the length of the flea.

$$field\ of\ view = \frac{16\ mm}{4} = 4\ mm$$

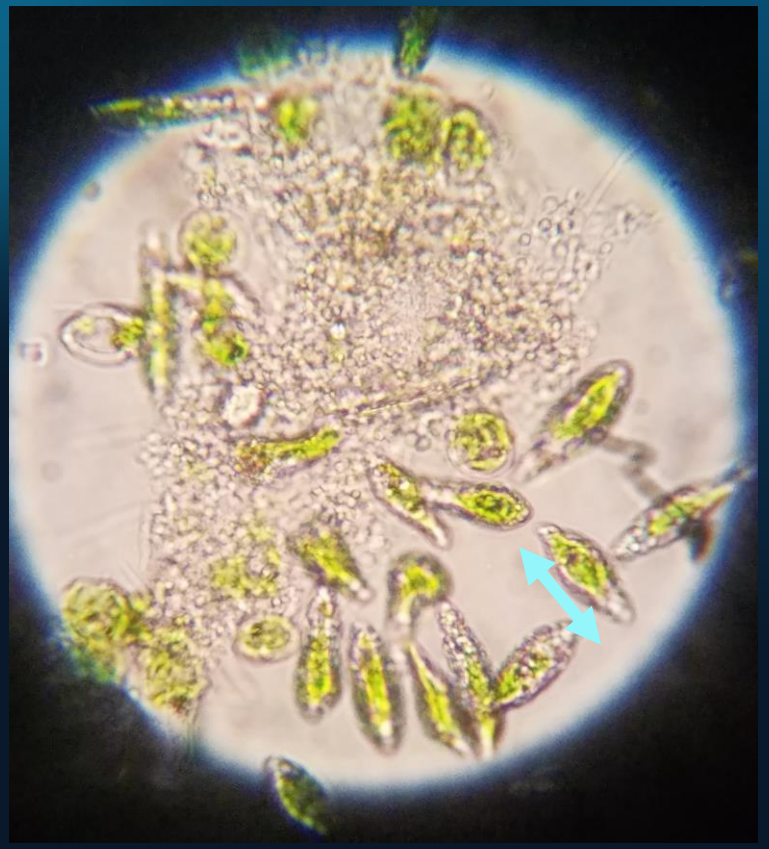
Estimate flea length to be 60% or 0.6 of the diameter of the field of view

$$flea\ length = 4\ mm \times 0.6 = 2.4\ mm$$

Specimen Size: Euglena

Euglena is a unicellular, eukaryotic, photosynthetic organism. It moves by rotating its whiplike flagellum.

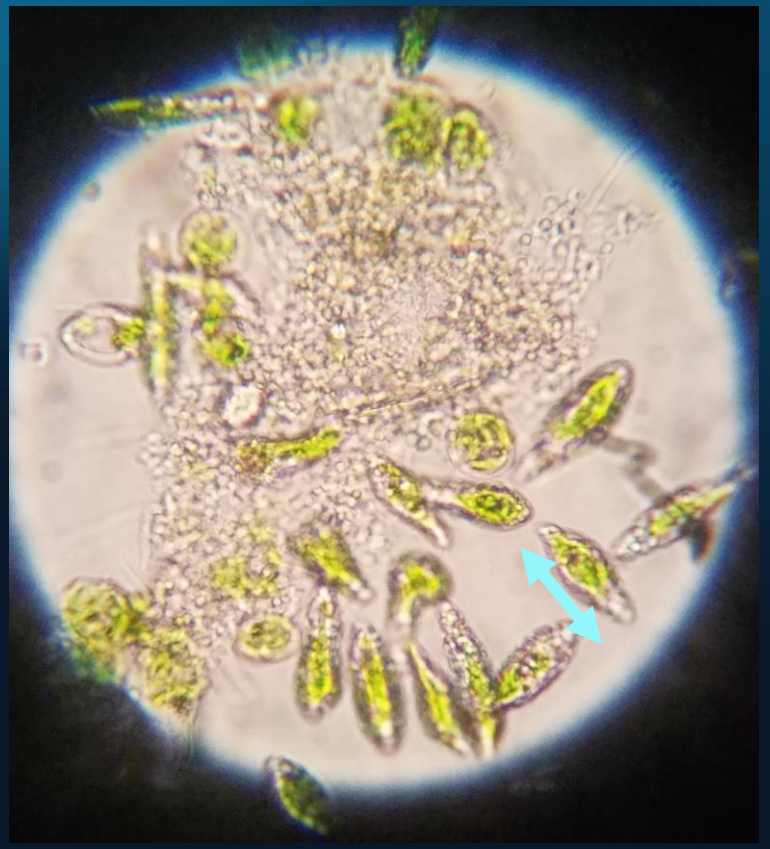
This image was taken using a 10x objective lens. Calculate the length of the labelled individual. Express your answer in micrometers.



Specimen Size: Euglena

$$field\ of\ view = \frac{20\ mm}{10} = 2\ mm$$

Estimate that 6 *Euglena* could fit across diameter; so *Euglena* is 1/6th the field of view.



$$length = 2\ mm \times \frac{1}{6} = 0.33\ mm$$

$$0.33mm \times \left(\frac{1000\mu m}{1mm}\right) = 330\ \mu m$$