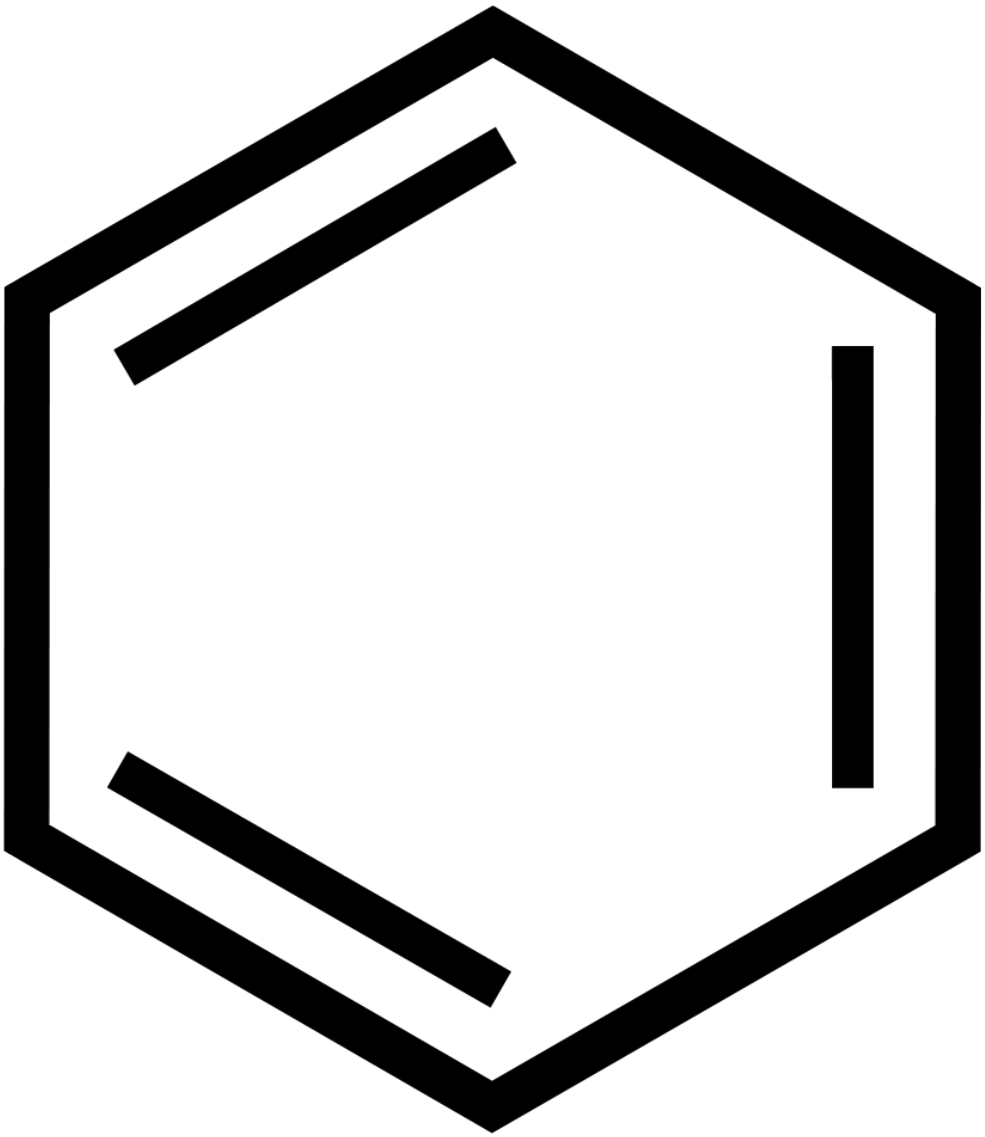

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CONCEPT: INTRODUCTION TO MICROSCOPES

●Microbes are simply too _____ for our eyes to see & require the aid of _____ to visualize them.

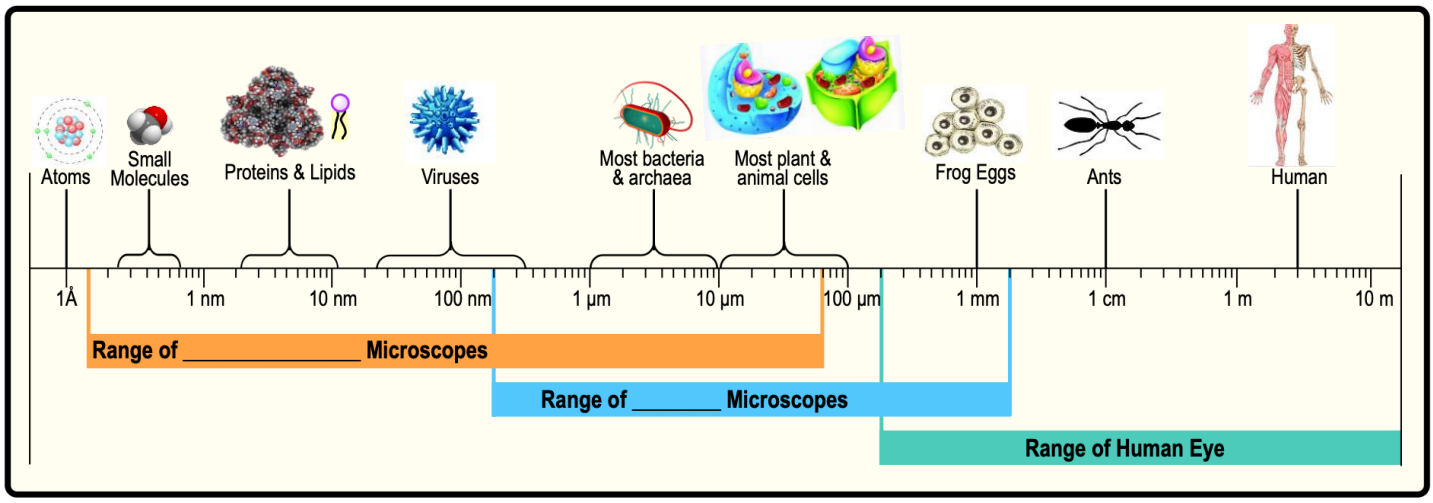
□ **Microscope:** an optical _____ used to visualize _____scopically *small* objects (ex. *cells*).

□ _____ main types of *microscopes* are commonly used:

1) **Light Microscopes**: uses visible _____ to magnify small objects.

2) **Electron Microscopes**: uses _____ for a *higher magnification* of even smaller objects.

EXAMPLE: Ranges of the Human Eye, Light Microscopes, & Electron Microscopes.



PRACTICE: The basic unit of life is a cell. What type of microscope can be used to visualize prokaryotic cells?

a) Light microscope.
b) Electron microscope.
c) Both light and electron microscopes.
d) None of the above, cells can be seen with the human eye.

PRACTICE: Which type of microscope would be the BEST selection for the examination of muscle proteins?

a) Light microscope.
b) Electron microscope.
c) No microscope would be needed to visualize muscle proteins.

CONCEPT: MAGNIFICATION, RESOLUTION, & CONTRAST

●Effective microscopy requires a balance of the following _____ terms:

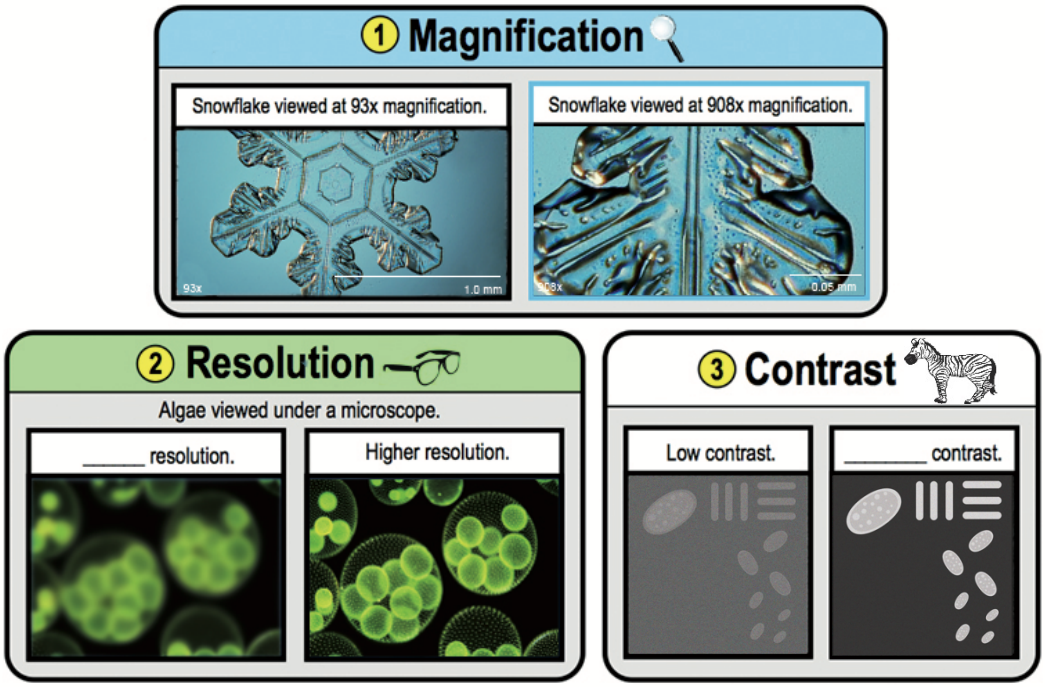
1) **Magnification**: an apparent increase in the size of an image through the use of _____.

2) **Resolution**: the _____ *distance* two objects must be to observe them as being *separate*.

□ **Resolving Power**: a measure of the ability to *distinguish* _____ objects that are very close together.

3) **Contrast**: *difference* in *color/light intensity* between an object and its _____.

□ Determines how easily cells & cell structures can be seen.



PRACTICE: The resolving power of a microscope is described as the ability of the microscope to…

a) Visually separate two objects that are very close together.

b) Magnify an object.

c) Differentiate the colors of the specimen from the background.

d) See structures at various depths in a tissue.

PRACTICE: If you can only increase one of the following, which would you increase to observe more details of the microscopic specimen?

a) Increase the magnification.

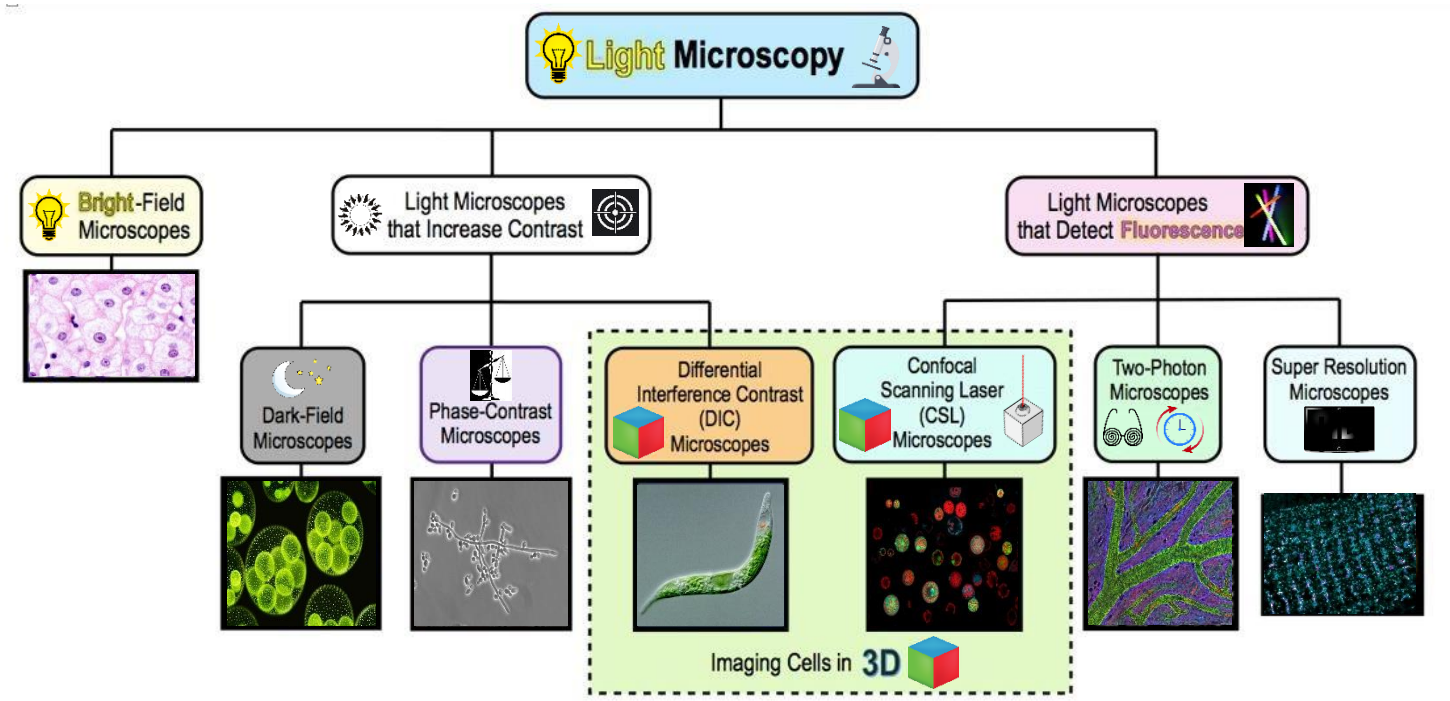
b) Increase the resolution.

CONCEPT: INTRODUCTION TO LIGHT MICROSCOPY

●**Light Microscopy**: uses visible _____ and lenses to magnify objects up to about 1000X.

□ There are _____ different types of light microscopes, each with their own set of advantages.

EXAMPLE: Outline of the light microscopy lesson.



PRACTICE: There are three major classes of light microscopes. Which class of light microscopes specializes in visualizing the difference in color intensity between the specimen and its background?

a) Bright-field microscopes.
b) Light microscopes that detect fluorescence.
c) Light microscopes that increase contrast.

PRACTICE: Some light microscopes are able to create 3-dimensional images of the microscopic specimen. Which class(es) of light microscopes are the 3-D microscopes found in?

a) Bright-field microscopes.
b) Light microscopes that increase contrast.
c) Light microscopes that detect fluorescence.
d) Both A and B.
e) Both B and C.
f) Both A and C.

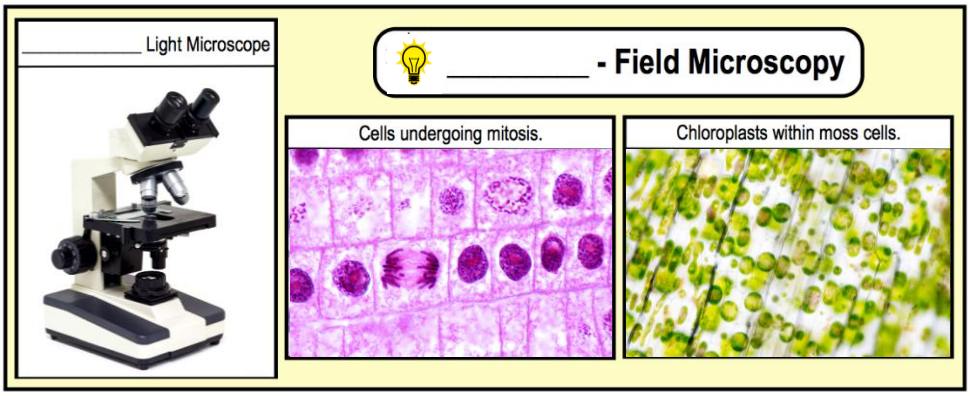
CONCEPT: LIGHT MICROSCOPY: BRIGHT-FIELD MICROSCOPE

●**Bright-Field Microscope**: the *most common* type of light microscope that generates a _____ background.

□ Routinely used to examine both *stained* & *unstained* specimens, forming a darker image on a lighter background.

□ *Drawback*: unstained transparent organisms create _____ contrast (staining procedures may kill organisms).

□ ***Compound* Light Microscope**: one of the most commonly used bright-field microscopes using _____ lenses.

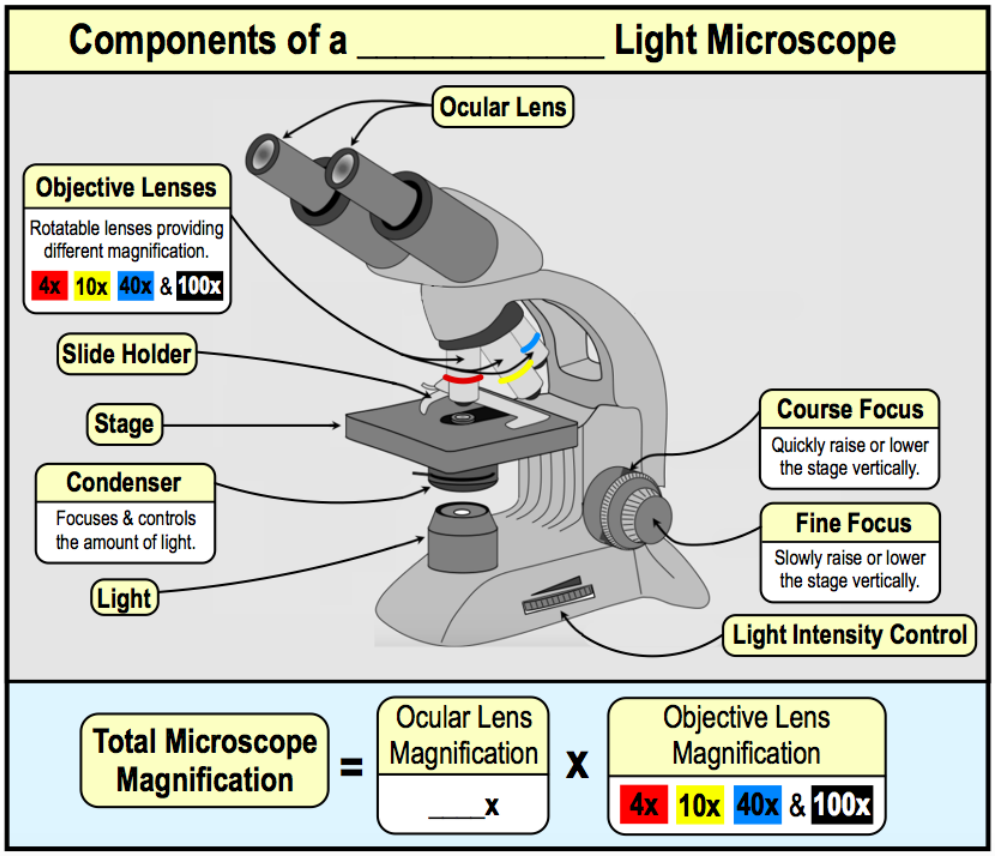


Components & Magnification of the Compound Light Microscope

●The compound light microscope has *several* components that you likely need to be familiar with.

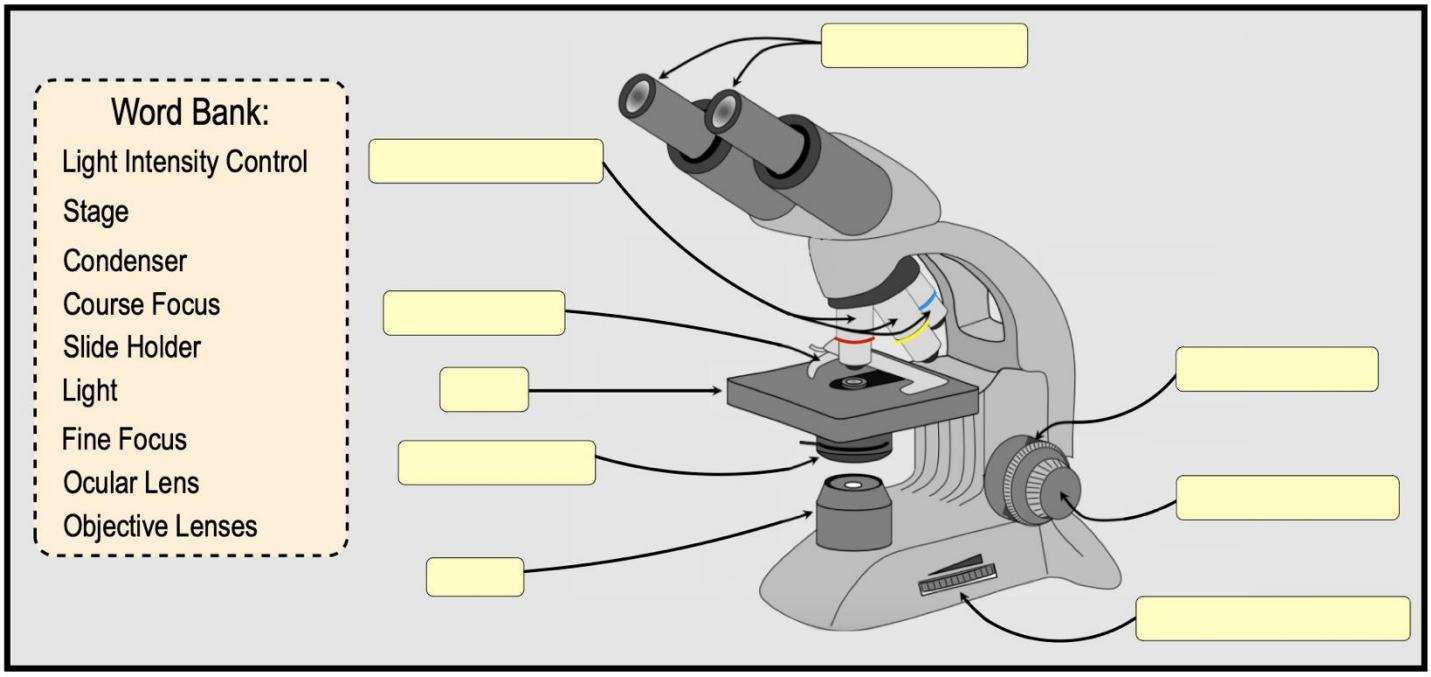
□ Light passes via _____ lenses (objective lens & ocular lens), each which provides additional magnification.

□ **Total magnification** of the specimen is the _____ of the magnification provided by each lens.



CONCEPT: LIGHT MICROSCOPY: BRIGHT-FIELD MICROSCOPE

EXAMPLE: Complete the following diagram by labeling each part of the compound microscope:



PRACTICE: The two magnifying lenses found in a light microscope are the:

a) Basic and transverse lenses.
b) Small and large lenses.
c) Ocular and objective lenses.
d) Simple and phase lenses.

PRACTICE: What is the purpose of the condenser on a light microscope?

a) Allows the viewer to change light intensity.
b) Focuses the light beam and controls the amount of light hitting the specimen.
c) Moves the microscope slide from left to right.
d) Focuses the image magnified by the objective lens.
e) Magnifies the microscopic specimen up to 100x.

CONCEPT: LIGHT MICROSCOPY: BRIGHT-FIELD MICROSCOPE

PRACTICE: What is the most common type of light microscope? And how does it work?

a) Dark-field microscope; shows brightly lit specimens on a dark background.
b) Light-field microscope; shows brightly lit specimens on a dark background.
c) Bright-field microscope; shows a darker specimen on a brightly lit background.
d) None of the above.

PRACTICE: What is the major drawback to bright-field microscopes?

a) Unstained microorganisms that are transparent are very difficult to see with bright-field microscopes.
b) Bright-field microscopes cannot be used to examine stained microorganisms.
c) The intensity of the light used to create the bright background in bright-field microscopes cannot be changed.
d) Bright-field microscopes are some of the most expensive microscopes used to visualize microorganisms.

PRACTICE: The knobs used for focusing the image on a compound light microscope are the:

a) Fine & capture knobs.
b) Course & tip knobs.
c) Stage & course knobs.
d) Course & fine knobs.

PRACTICE: Which part of a compound microscope has the shutter that focuses & controls the amount of light?

a) The condenser.
b) The eyepiece.
c) The stage.
d) Fine adjustment knob.

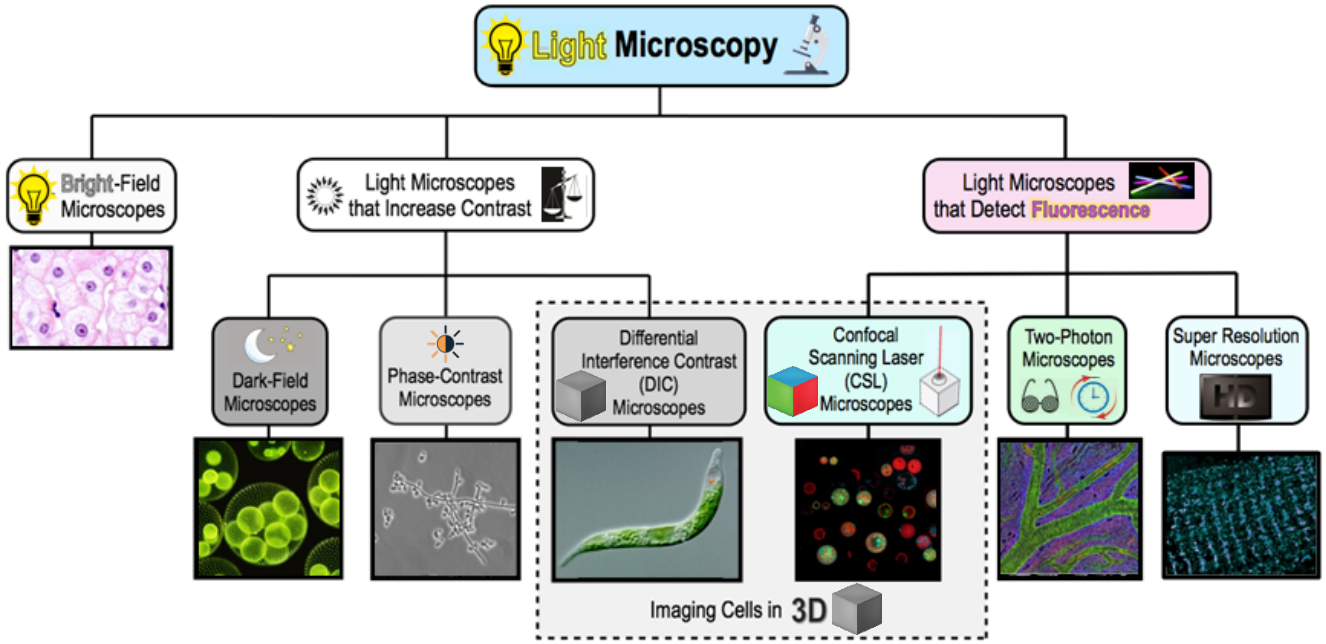
CONCEPT: LIGHT MICROSCOPES THAT INCREASE CONTRAST

●Recall: a drawback of brightfield-light microscopy is visualizing unstained transparent organisms due to poor contrast.

□ Staining cells with dyes can increase contrast but it can also kill cells and/or distort their features.

●Other *special types of light microscopes* help *improve* _____ when visualizing unstained cells:

□ _____-Field Microscopes. □ _____-Contrast Microscopes. □ _____ Microscopes.

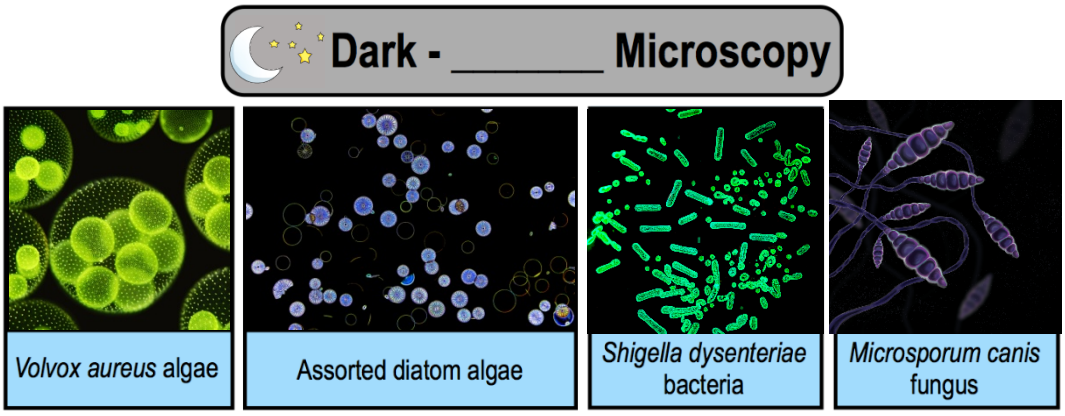


c

Dark-Field Microscopy

●_____**-Field Microscopes**: observe bright specimens against a _____ background.

□ A special mechanism directs light at an angle so that only light scattered by the specimen is observed.



PRACTICE: What type of microscopy uses a special condenser that only allows light scattered by the specimen to reach the objective lens and causes the specimen to appear bright?

a) Phase-contrast microscopy.

b) Differential interference contrast (DIC) microscopy.

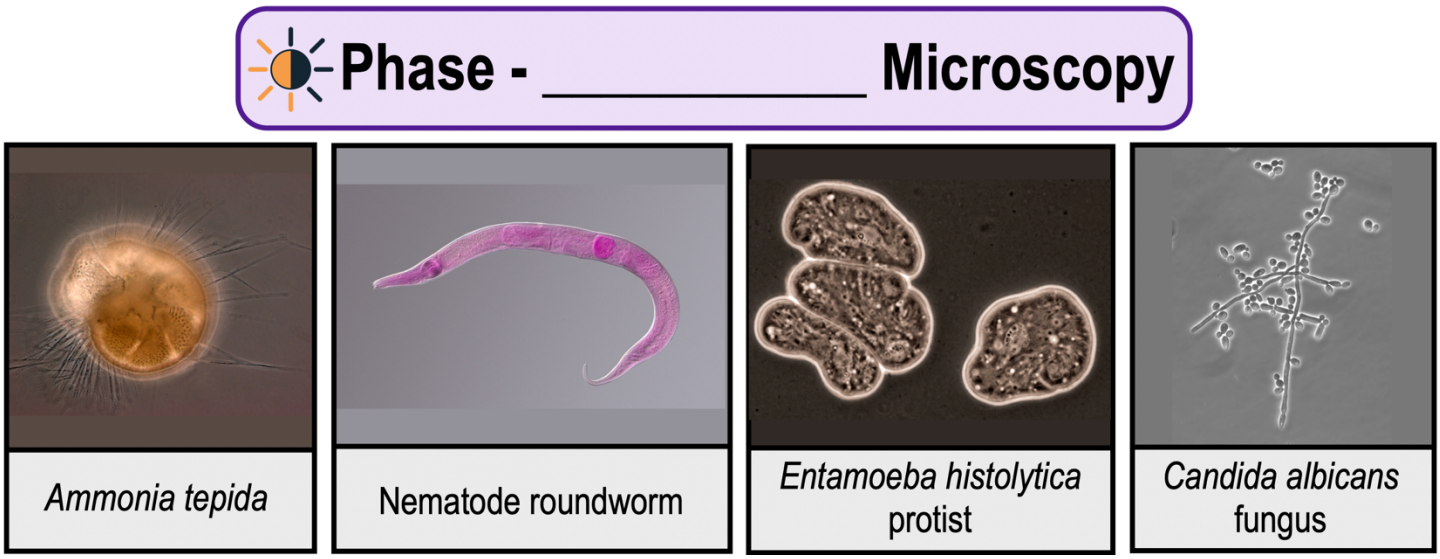
c) Dark-field microscopy.

CONCEPT: LIGHT MICROSCOPES THAT INCREASE CONTRAST

Phase-Contrast Microscopy

- _____**-Contrast Microscopes**: makes cells & their dense structures appear darker than the gray background.
  - □ Special optic devices increase the differences in how different substances/surroundings refract light differently.
  - □ Excellent for enhancing contrast of _____ details in unstained, living cells.

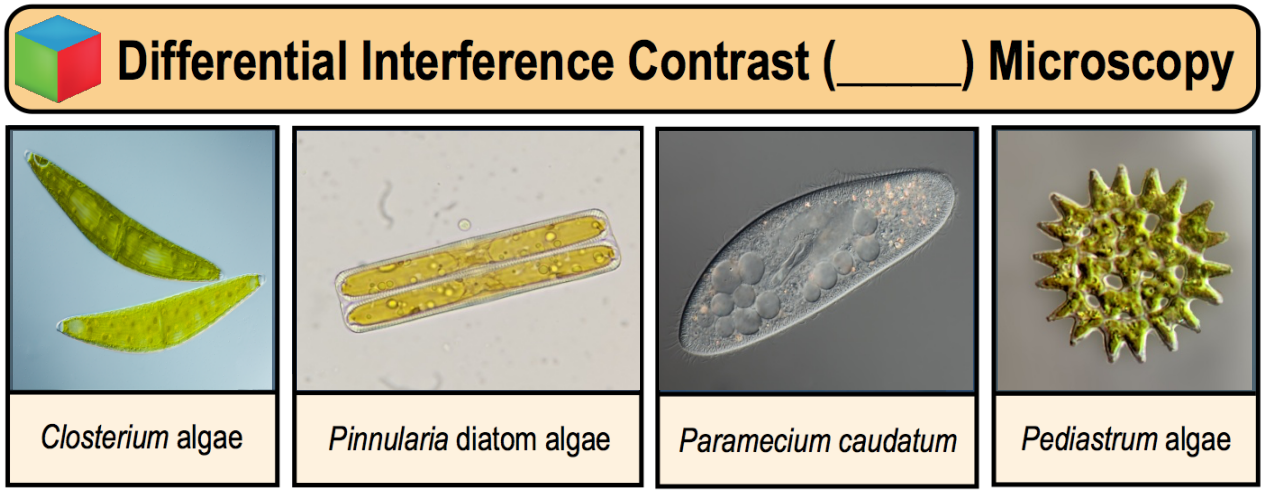
EXAMPLE: Images from a Phase-Contrast Microscope.



Differential Interference Contrast (DIC) Microscopy

- _____ **Microscopes**: creates very detailed, highly contrasting, _____-images of live specimens.
  - □ Similar to phase-contrast microscopy, but different mainly by adding contrasting *colors* & creating *3D*-images.

EXAMPLE: Images from a Differential Interference Contrast (DIC) Microscope.



CONCEPT: LIGHT MICROSCOPES THAT INCREASE CONTRAST

PRACTICE: This form of microscope is able to refract light off of the dense structures of a cell making them appear brighter than the gray background.

a) Phase-contrast microscope.
b) Bright-field microscope.
c) Differential interference contrast microscope.
d) Dark-field microscope.

PRACTICE: The microscope that increases contrast and allows the specimen to appear three-dimensional is the…

a) Phase contrast microscope.
b) Differential interference contrast microscope.
c) Fluorescence microscope.
d) Dark-field microscope.

PRACTICE: Which of the following microscope types would be least useful in viewing unstained living cells?

a) Phase-contrast microscope.
b) Differential interference contrast microscope.
c) Bright-field microscope.
d) Dark-field microscope.

PRACTICE: Which type of microscopes use light that hits the specimen indirectly, producing a darker image of the specimen on a brighter background?

a) Phase-contrast microscope.
b) Differential interference contrast microscope.
c) Bright-field microscope.
d) Dark-field microscope.

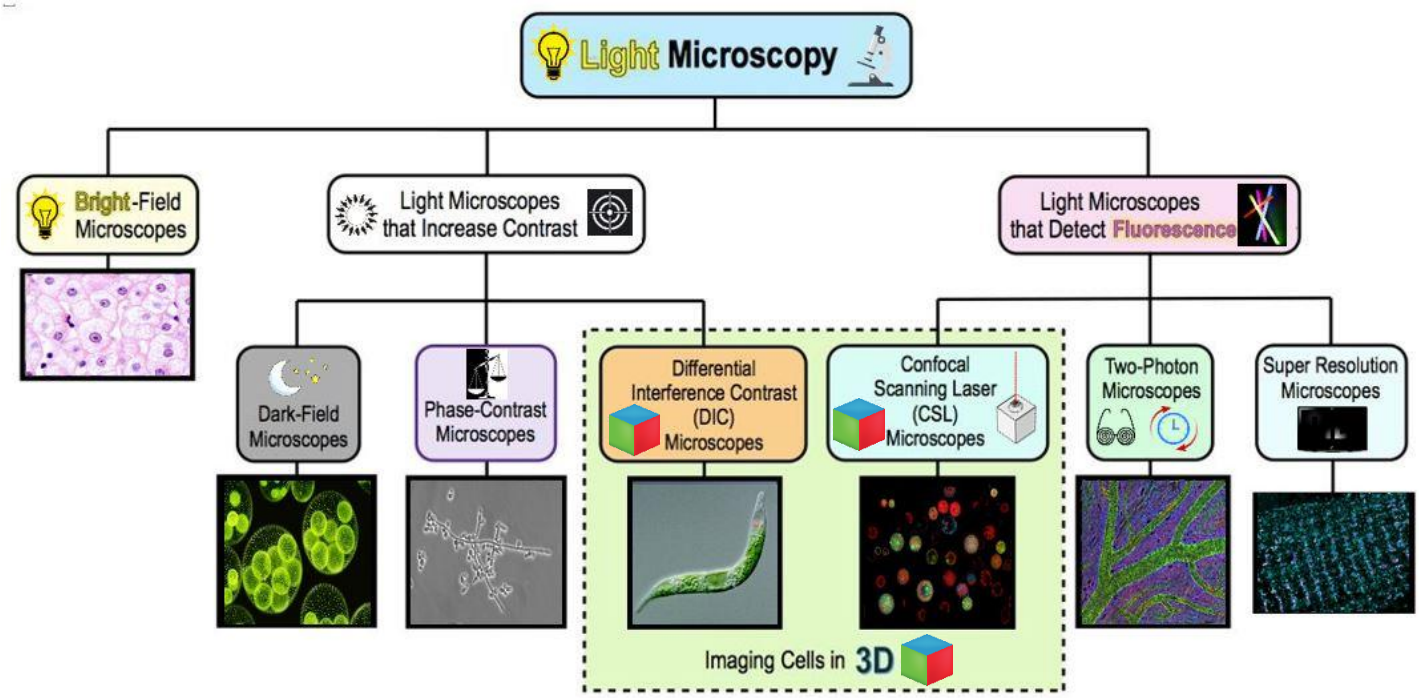
CONCEPT: LIGHT MICROSCOPES THAT DETECT FLUORESCENCE

●In certain situations, light microscopes that detect _____ (emitted light) can be useful.

□ **Fluorescence**: ability to absorb short wavelengths of light & give off longer wavelengths of _____ light.

□ Fluorescent molecules stand out as _____ objects against a dark background.

□ Several different types of microscopes can detect fluorescence.



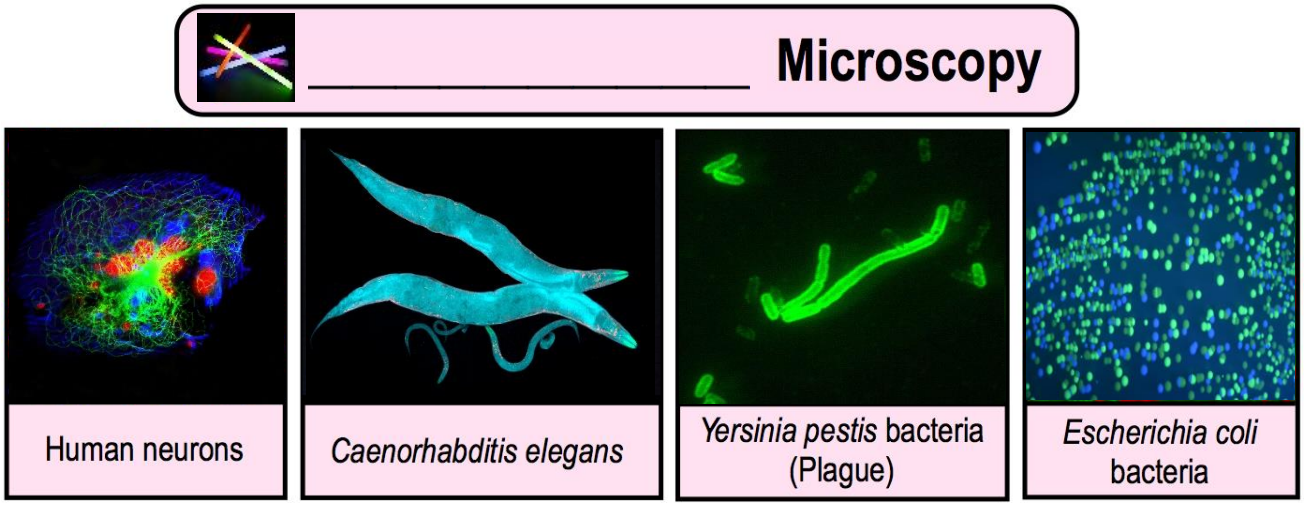
Fluorescence Microscopes

●**Fluorescence Microscopes**: light microscope that projects ultraviolet light onto the specimen, causing it to fluoresce.

□ Some organisms fluoresce *naturally*, but scientists can tag molecules with *fluorescent* _____ (*fluorochromes*).

□ **Immunofluorescence**: technique combining a *fluorochrome* with an _____ to tag specific objects.

EXAMPLE: Fluorescence Microscopy.

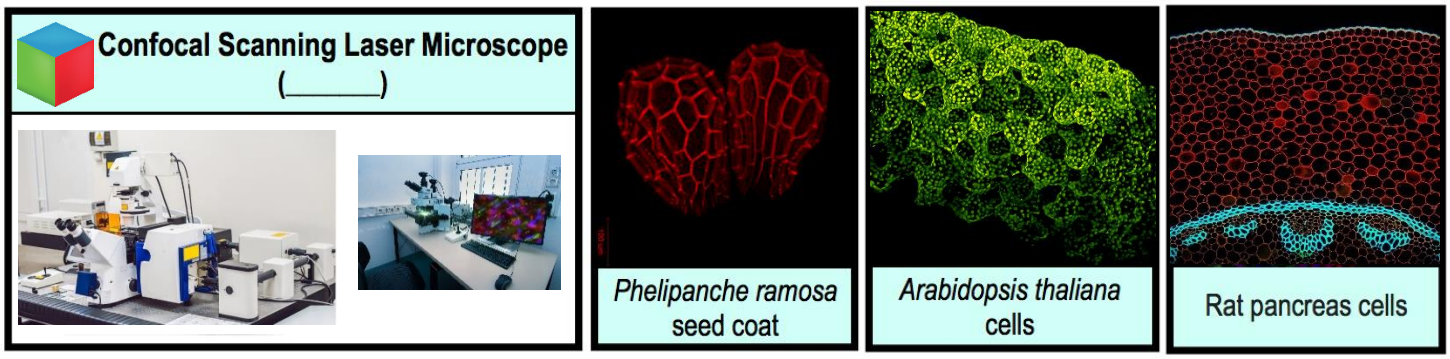


CONCEPT: LIGHT MICROSCOPES THAT DETECT FLUORESCENCE

Confocal Scanning Laser Microscopes (CSLMs)

- _____: *computer-controlled* microscopes that couples a *laser* to a *fluorescence* microscope.
  - □ *Laser* generates high-contrast, _____-image, allowing viewer to access several planes of focus in the specimen.
  - □ Can be used to look inside cells at different layers.

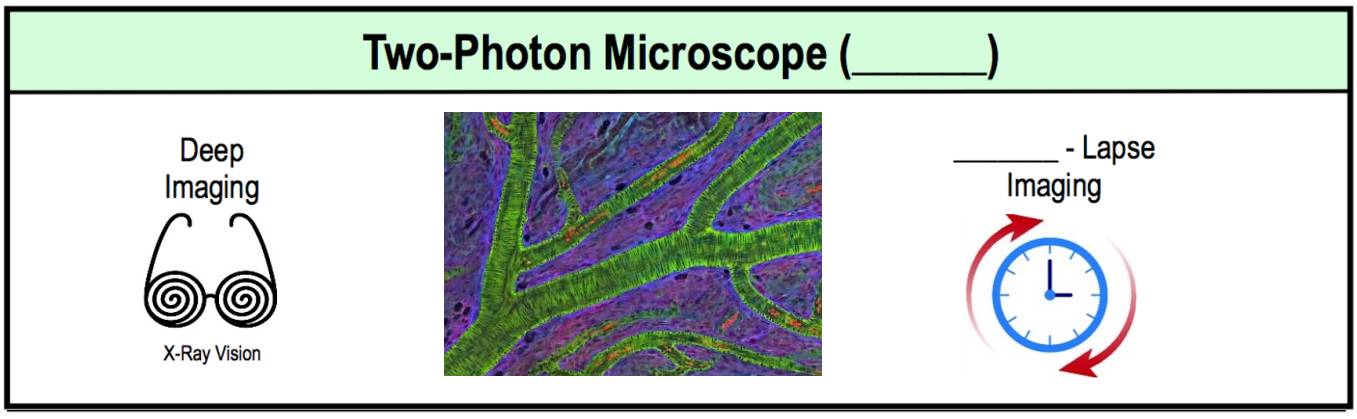
EXAMPLE: Images from a Confocal Scanning Laser Microscope (CSLM).



Two-Photon Microscope (TPM)

- _____**-Photon Microscope (TPM)**: similar to CSLM, except it uses _____ photons & less damaging light.
  - □ Also known as _____*-photon microscopy*.
  - □ Longer, less-damaging light wavelengths allow for *deeper* imaging of _____ structures & *time-lapse* imaging.

EXAMPLE: Functions of a Two-Photon Microscope (TPM).



PRACTICE: Which type of light microscope can show the movement of cells or structures in living specimens with time-lapse imaging?

a) Confocal scanning laser microscope.
b) Fluorescence microscope.
c) **Two-photon microscope.**
d) Differential interference contrast microscope.

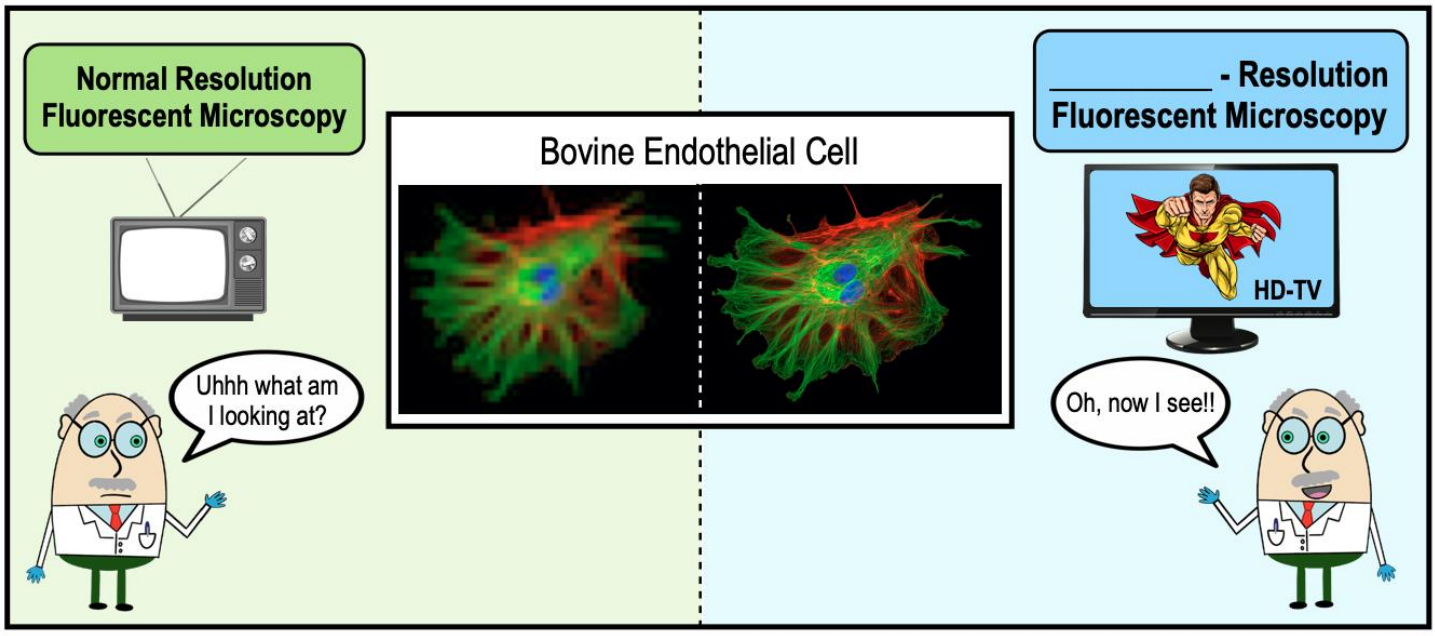
CONCEPT: LIGHT MICROSCOPES THAT DETECT FLUORESCENCE

Super-Resolution Microscope (SRM)

●Up until 2014 when *super-resolution microscopes* were developed, the max resolution for light microscopes was ~0.2 µm.

- □ _____**-Resolution Microscope (SRM)**: *fluorescence* light microscope with high resolution (~0.01 µm).
- □ Uses complex mechanisms to visualize molecules that otherwise are too _____ together to be seen as distinct.
- □ Sometimes can allow for even a single _____ to be tracked.

EXAMPLE:



PRACTICE: Which type of fluorescent, light microscope creates high-contrast, 3D-images that allow the viewer to access several planes of focus in the specimen?

a) Super-resolution microscope.
b) Confocal scanning laser microscope.
c) Two-photon microscope.
d) Immunofluorescent microscope.

PRACTICE: New and innovative technology, known as _____ microscopes, allow scientists to fluorescently tag and track single molecules in a living cell.

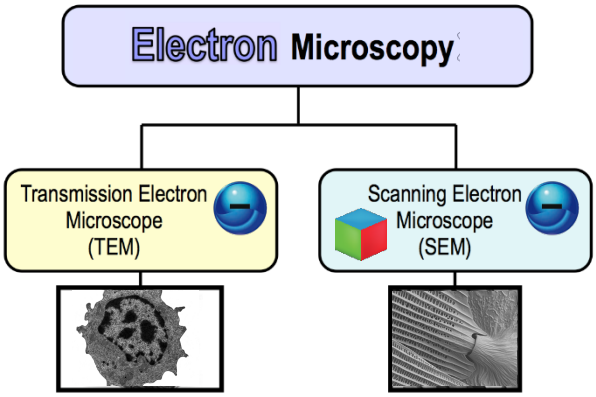
a) Two-photon microscopes.
b) Confocal scanning laser microscopes.
c) Super scanning laser microscopes.

Super-resolution microscopes.

CONCEPT: ELECTRON MICROSCOPES

●Recall: _____ microscopes use *electrons* & are more powerful than light microscopes.

- □ Provide greater _____ up to 10,000,000X (in comparison to just 1000X in light microscopes).
- □ Provides greater _____ of ~0.3 nm (in comparison to 10 nm in a super-resolution light microscope).
- □ Complex instruments & specimen preparation that limits observation to only _____-living cells/objects.
- □ Electron micrograph images are *black-&-white* but can be falsely _____ with computers.

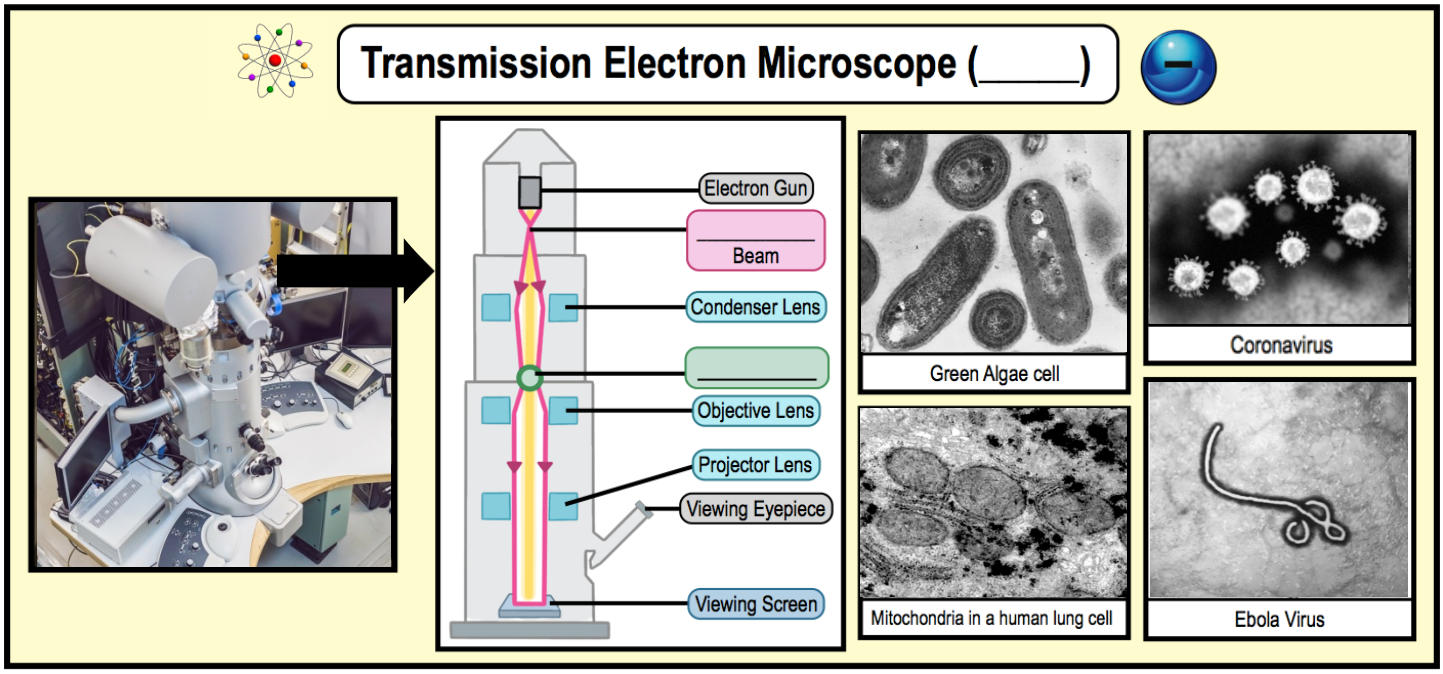


Transmission Electron Microscope (TEM)

●**Transmission Electron Microscope (_____)**: form a 2D-image from a beam of electrons *passing through* a specimen.

- □ A variety of sample preparations may be used, but many used for visualizing _____ cell structures.
- □ Specimens must be viewed in a vacuum and in extremely thin slices of just 20 to 100 nm.
- □ *Drawback:* sometimes a complex preparation can distort cells & cause artificial artifacts to form.

EXAMPLE: Diagram of a Transmission Electron Microscope (TEM).



CONCEPT: ELECTRON MICROSCOPES

PRACTICE: Electron microscopes differ from light microscopes in that:

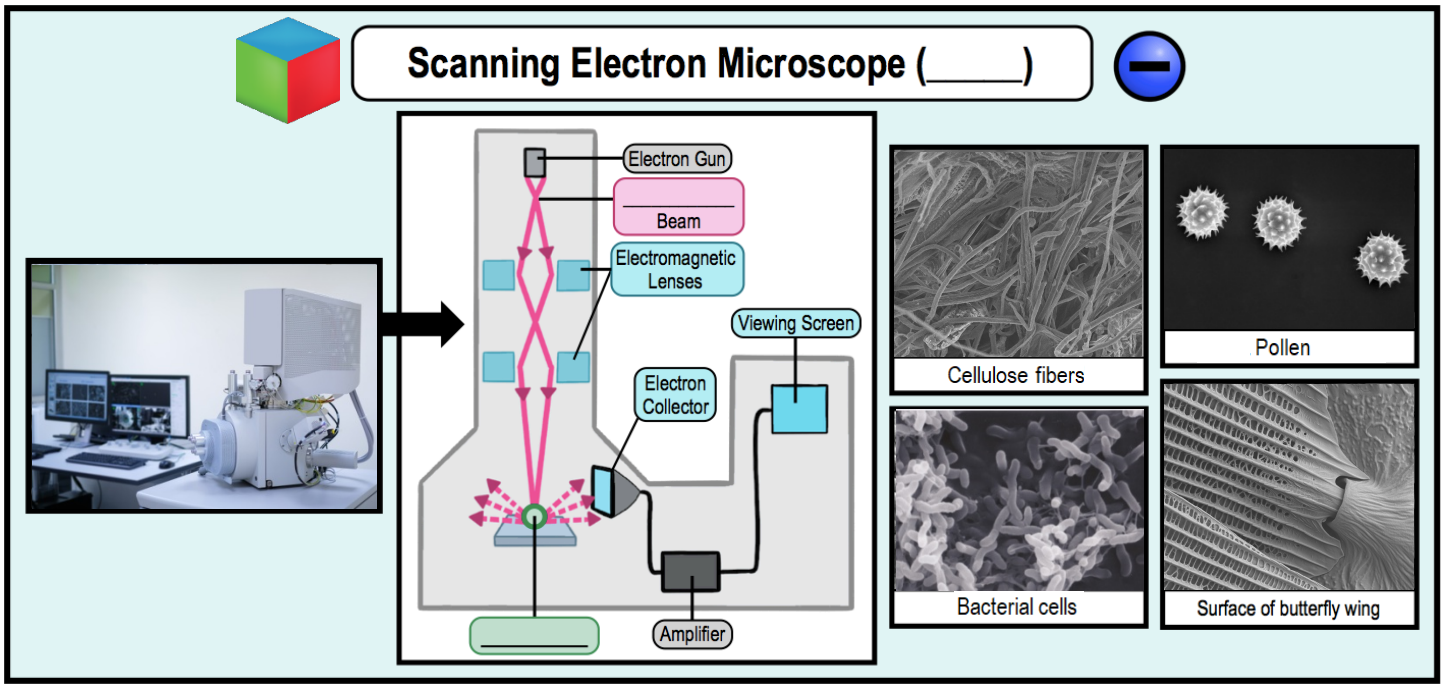
a) Electron microscopes use an electron beam instead of a light beam.
b) Electron microscopes can magnify the specimen significantly more than light microscopes.
c) Electron microscopes have significantly higher resolution than light microscopes.
d) All of the above.

Scanning Electron Microscope (SEM)

●**Scanning Electron Microscope (_____)**: forms 3D-image from beam of electrons *scattering off* a specimen's surface.

□ A variety of sample preparations may be used for visualizing _____ cell structures on the surface.

EXAMPLE: Diagram of a Scanning Electron Microscope (SEM).



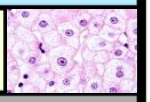
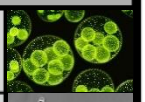
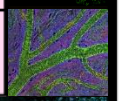
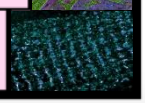
PRACTICE: There are two major types of electron microscopes, Transmission Electron Microscopes (TEM) & Scanning Electron Microscopes (SEM). What are the major differences between these types of electron microscopes?

a) TEM shows 2D images of the specimen while SEM shows 3D images of the specimen.
b) SEM specimen preparations can damage or distort the microorganism's features, while TEM preparations do not.
c) TEM shows internal cell structures, while SEM show external cell structures.
d) Both A and B.
e) Both B and C.
f) Both A and C.

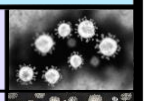
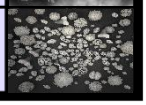
CONCEPT: REVIEWING THE DIFFERENT TYPES OF MICROSCOPES

x1w● Now let's review the different types of light & electron microscopes.

Light Microscopes

Type of Microscope	Description	
_____- Field Microscope	Observe stained or unstained specimens on a bright background.	
Light Microscopes that Increase Contrast		
_____- Field Microscope	Observe bright specimens against a dark background.	
Phase - _____ Microscope	Cells & their dense structures appear darker than the gray background.	
Differential Interference Contrast (_____) Microscope	Very detailed, highly contrasting, 3D-images of live specimens.	
Light Microscopes that Detect Fluorescence		
Confocal Scanning _____ (_____) Microscope	High-contrast, 3D-image showing several planes of focus in the specimen.	
Two - _____ Microscope	High-contrast, 3D-images of deep structures. Time-lapse images.	
_____ Resolution Microscope	Light microscope with extremely high resolution (~0.01 µm).	

Electron Microscopes

Type of Microscope	Description	
_____ Electron Microscope (TEM)	2D-image from a beam of electrons passing through a specimen.	
_____ Electron Microscope (SEM)	3D-image from beam of electrons *scattering off* a specimen's surface.	

CONCEPT: REVIEWING THE DIFFERENT TYPES OF MICROSCOPES

PRACTICE: Match the microscope with its function.

Types of Microscopes:

A. Confocal Scanning Laser (CSL) Microscope.
B. Bright-Field Microscope.
C. Phase-Contrast Microscope.
D. Transmission Electron Microscope (TEM).
E. Dark-Field Microscope.
F. Two-Photon Microscope.
G. Super Resolution Microscope.
H. Scanning Electron Microscope (SEM).
I. Differential Interference Contrast (DIC) Microscope.

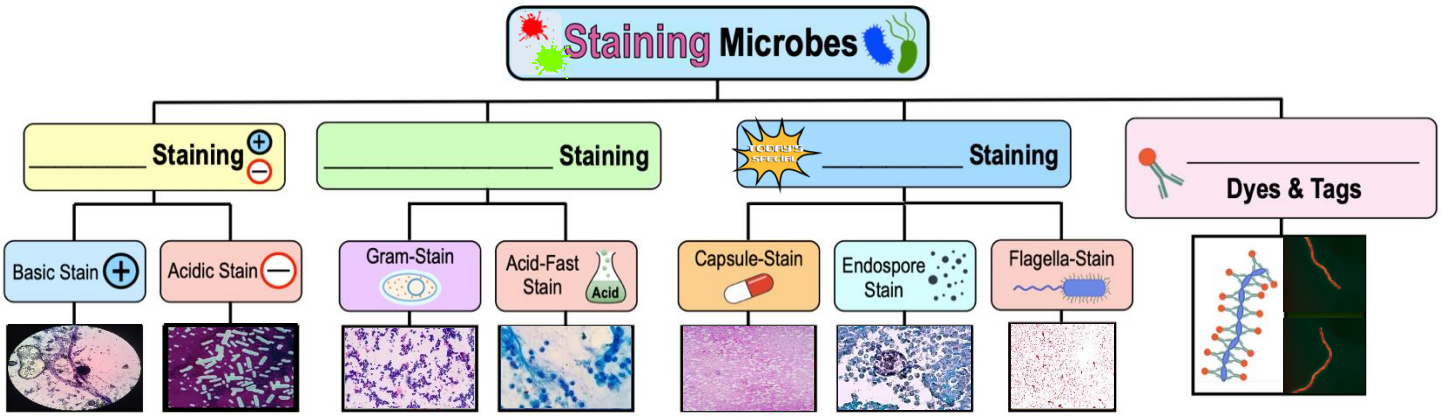
1. _____ Creates high contrast, 3D images of deep structures and time lapse images.
2. _____ Creates 2D images from a beam of electrons passing through a specimen.
3. _____ Creates images where the specimen's dense structures appear darker than the gray background.
4. _____ Allows the scientist to view stained or unstained specimens on a bright background.
5. _____ A light microscope with extremely high resolution.
6. _____ Creates 3D images from a beam of electrons scattering off a specimen's surface.
7. _____ Creates very detailed, high contrast, 3D images of live specimens.
8. _____ Allows the scientist to view specimens against a dark background.
9. _____ Creates high contrast, 3D images that show several planes of focus in the specimen.

CONCEPT: INTRODUCTION TO STAINING

●Recall: many unstained microbes are colorless & transparent, creating poor contrast, making them difficult to visualize.

□ _____ microbes with one or more dyes can help to improve contrast.

□ Several different types of stains/dyes & staining techniques can be used in different scenarios.



PRACTICE: Why is staining useful when viewing microbes under a microscope?

a) Staining allows the scientist to better visualize the environment behind the microbe.

b) Staining allows the scientist to increase the magnification of the microscope.

c) Staining allows the scientist to better visualize microbes which are usually colorless and transparent.

PRACTICE: Gram-Staining is what type of staining technique?

a) A special staining technique.

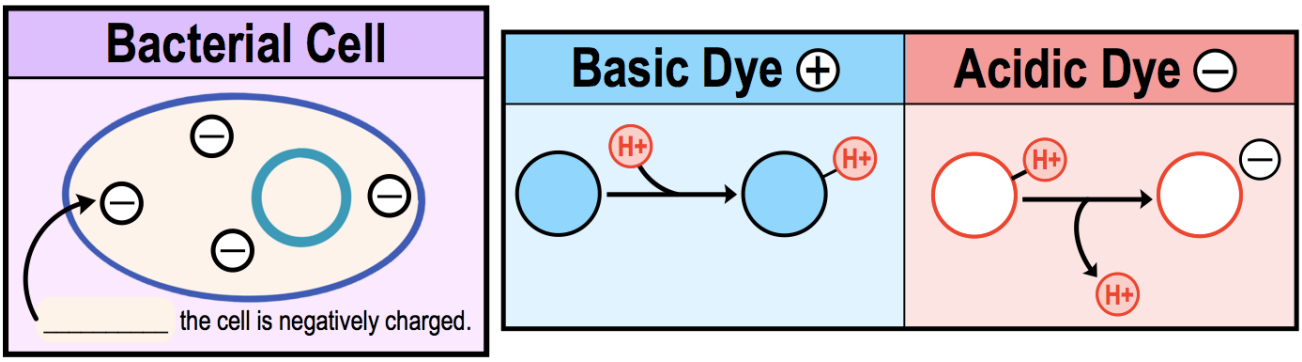
b) A differential staining technique.

c) A simple staining technique

d) A fluorescent staining technique.

CONCEPT: SIMPLE STAINING

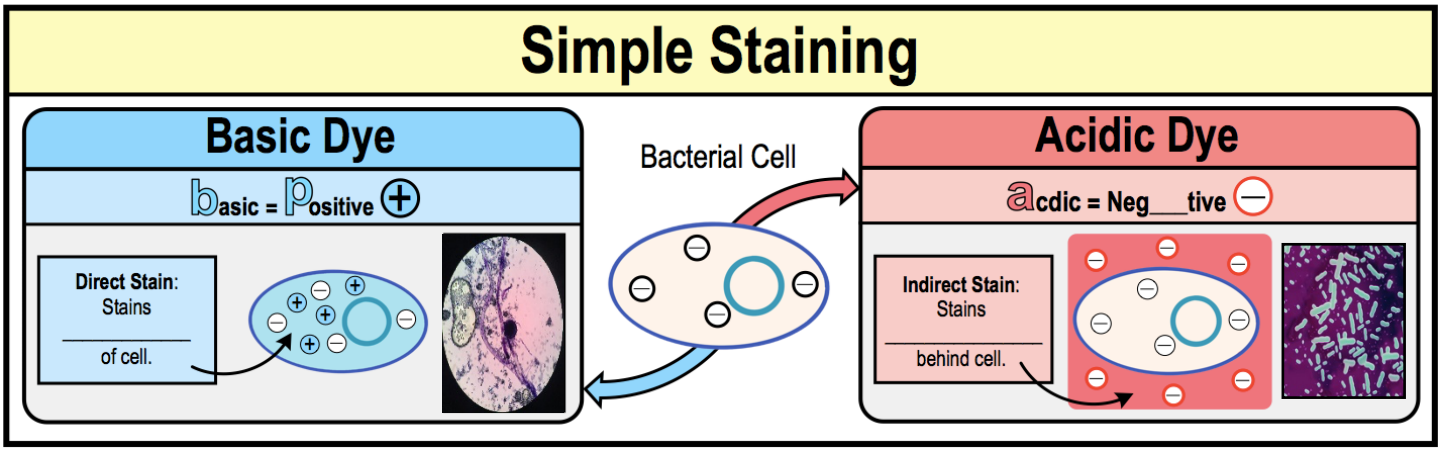
- _____ **Staining**: a *simple* staining procedure using only _____ *single* dye to stain a specimen.
- There are 2 important things to remember regarding simple stains:
  1) The *inside* of a bacterial cell is _____ charged with respect to the outside.
  2) The dye (*basic* or *acidic*) is only effective _____ it reacts.



Simple Staining Basic & Acidic Dyes

- There are _____ main types of dyes used in *simple* staining:
  1) _____ **Dye**: act as *base* to become _____ charged dye particles that stain inside *cells*.
     - □ Positively charged, basic dye is _____ to negatively charged cell components.
  2) _____ **Dye**: act as *acid* to become _____ charged dye particles that stain *backgrounds*.
     - □ Negatively charged, acidic dye _____ negatively charged cell components & color background.
     - □ **Negative Staining**: staining procedure using an *acidic* dye to stain _____.

EXAMPLE: Basic & acidic simple staining.



- Simple staining can increase the _____ of a *brightfield* microscope.

CONCEPT: SIMPLE STAINING

PRACTICE: There are two major types of simple stains used to better visualize microorganisms. What are these two types of simple stains and how do they differ?

a) Basic stain is a positively charged dye; Acidic stain is a neutral dye.
b) Basic stain is a positively charged dye; Acidic stain is a negatively charged dye.
c) Basic stain is an indirect stain; Acidic stain is a direct stain.

PRACTICE: Simple staining is a procedure often used to increase the contrast of _____ microscopes.

a) Dark-field microscopes.
b) Fluorescence microscopes.
c) Compound light (brightfield) microscopes.
d) Transmission electron microscopes.

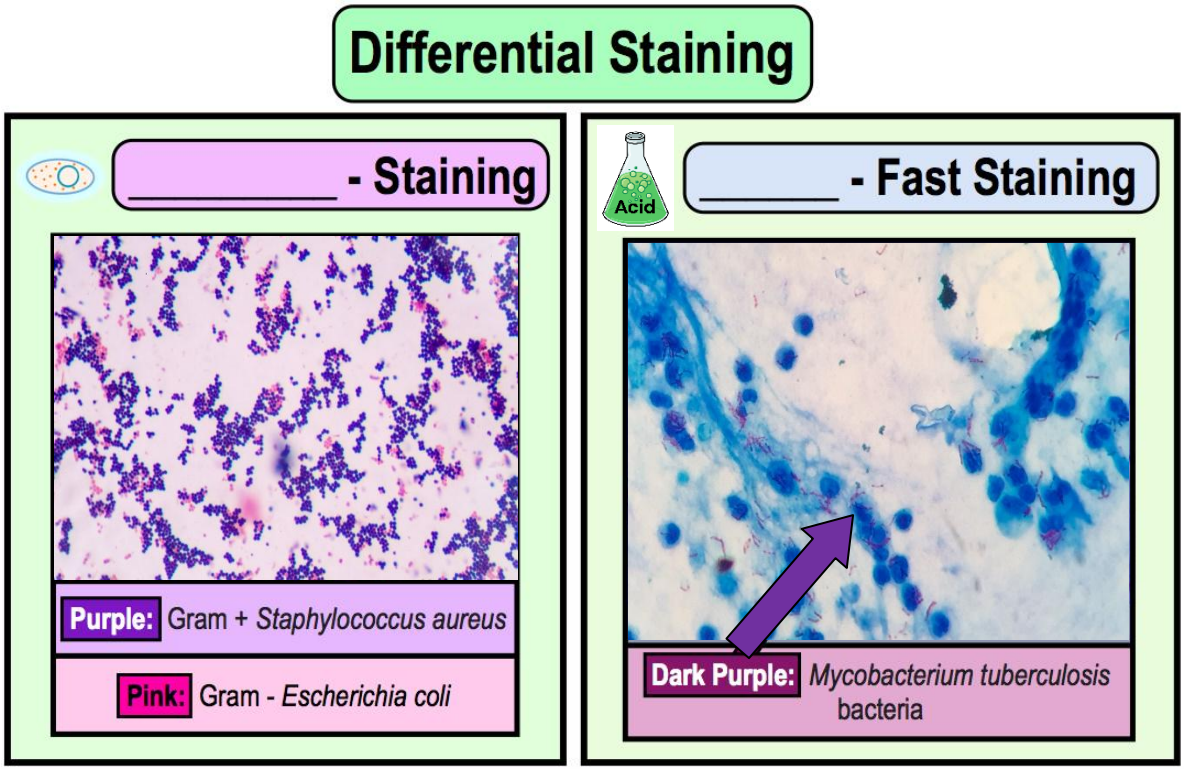
PRACTICE: Which of the following statements is true?

a) A basic dye is negatively charged & stains the outside of a cell while an acidic dye is positively charged & stains the inside of a cell.
b) A basic dye is positively charged & stains the inside of a cell while an acidic dye is negatively charged & stains the outside of a cell.
c) A basic dye is positively charged & stains the outside of a cell while an acidic dye is negatively charged & stains the inside of a cell.
d) A basic dye is negatively charged & stains the inside of a cell while an acidic dye is negatively charged & stains the outside of a cell.

CONCEPT: DIFFERENTIAL STAINING

- _____ **Staining**: uses multiple dyes to distinguish/*differentiate* different groups of bacteria.
  - □ Two most common examples are _____ stain & _____-*fast* stain.
  - □ **Gram-Stain**: differentiates bacteria based on differences in the cell _____ (gram-positive vs gram-negative).
  - □ **Acid-Fast Stain**: identifies acid-fast bacteria with a _____ material (mycolic acid) in their cell walls.

EXAMPLE: Types of differential staining.



PRACTICE: A scientist is examining more than one species of bacteria under a microscope at the same time. The scientist decides to differentiate the bacterial cells based on their cell wall/cell envelope structure. Which staining technique should she use?

a) Gram-staining.
b) Basic staining.
c) Envelope staining.
d) None of the above.

PRACTICE: A scientist has a sample containing a variety of different bacteria species. She wishes to identify which bacteria in her sample are of the genus Mycobacterium. Mycobacterium have a wax-like, nearly impermeable cell wall which contains mycolic acid. Which type of staining technique should the scientist use to identify the Mycobacterium?

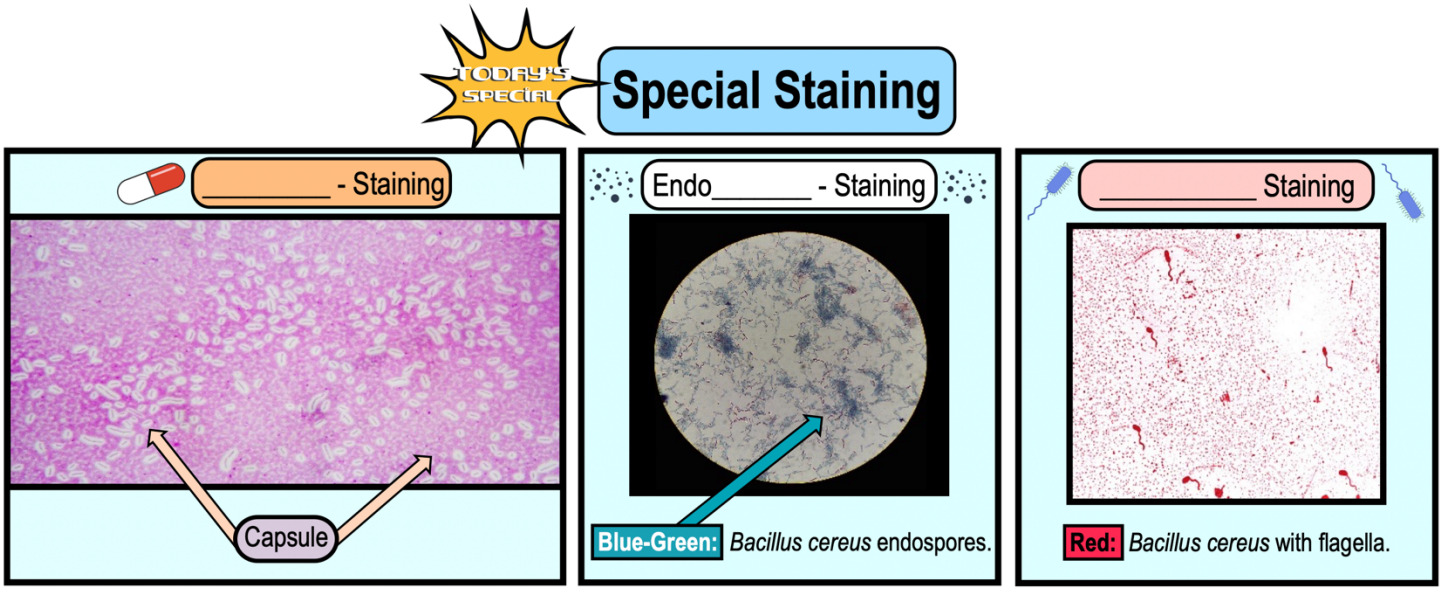
a) Basic staining.
b) Gram-staining.
c) Mycolic staining.
d) Acid-fast staining.

CONCEPT: OTHER TYPES OF STAINING

Special Staining

- _____ **Staining**: special procedures used to stain very specific structures inside or outside cells.
  - Examples include *capsule* stain, *endospore* stain & _____ stain.

EXAMPLE: Types of special staining.



PRACTICE: Which of these is considered a special stain that correctly matches its description?

a) Endospore stain: stains the capsules of bacteria found in the human eye.
b) Gram-stain: used to differentiate gram-negative from gram-positive bacteria.
c) Acid-fast stain: identifies acid-fast bacteria that contain mycolic acid in their cell walls.
d) Flagella stain: stains the flagellum of bacteria like *Bacillus cereus.*

PRACTICE: What is the major difference between special staining techniques and differential staining techniques?

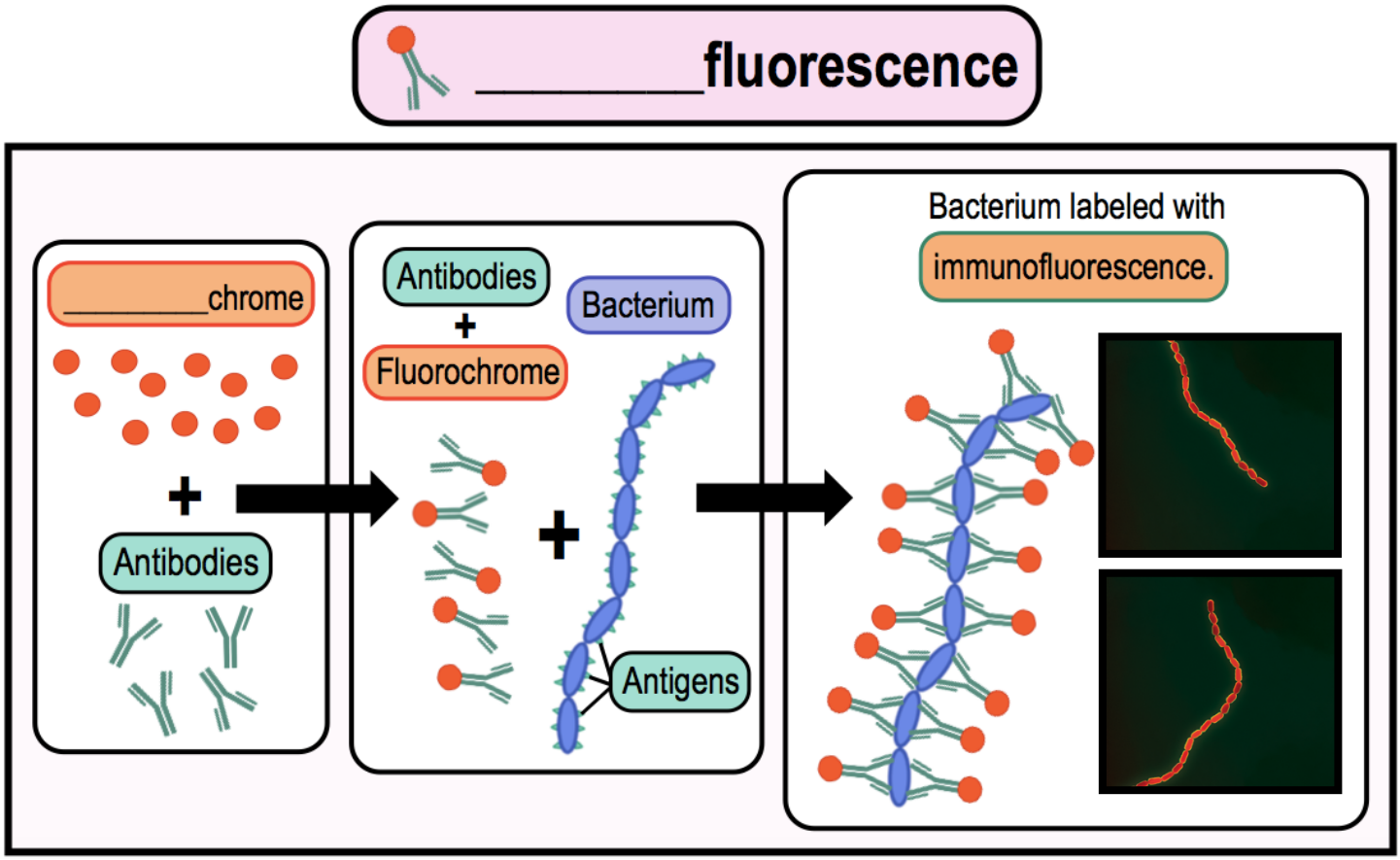
a) Special staining stains the background behind the specimen. Differential staining stains the specimen.
b) Special staining stains specific structures of a cell. Differential staining differentiates different type of specimens.
c) Special staining differentiates gram + from gram – cells. Differential staining stains capsules and flagella.

CONCEPT: OTHER TYPES OF STAINING

Fluorescent Dyes

●Recall: _____ dyes & *immunofluorescence* can be used to observe cells &/or cell components.

- □ **Immunofluorescence**: technique combining a *fluorochrome* with an _____ to tag specific objects.
- □ Some fluorescent dyes can be changed by cellular processes to distinguish *living* from *dead* cells.

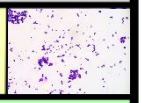
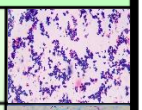
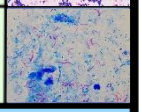
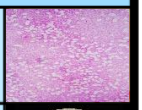
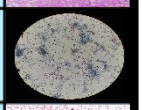
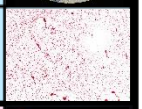
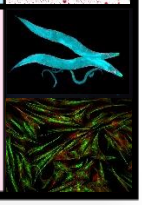


PRACTICE: _____ is/are used to attach fluorochrome molecules to antigens on the surface of bacterial cells in immunofluorescence.

a) DNA.
b) Ribosomes.
c) Antibodies.
d) Flagella.

CONCEPT: REVIEWING THE TYPES OF STAINING

●Now let's review the types of staining:

Type of Stain	Description	
Simple Stains ⊕ ⊖	A simple dye which stains cells or the _____ behind cells.	
Differential Stains	A procedure that stains different microorganisms different colors.	
_____ Stains	A procedure that stains Gram + and Gram - bacteria cells different colors.	
Acid-Fast Stains	A dye used to stain microorganisms that are not easily stained.	
Special Stains	A procedure that stains specific cell structures.	
_____ Stain	A procedure that stains the background so the capsules of cells visually stand out.	
Endospore Stain	A special dye used to stain endospores which normally do not stain.	
Flagella Stain	A special dye that coats the outside of the _____ making it more visible.	
Fluorescent Dyes & Tags	**Fluorescent Dyes:** Dyes which can be made to stain all cells or only specific cell structures. **Fluorescent Tags:** Antibodies with an attached fluorescent _____ which stain specific molecules.	

PRACTICE: Which of the following stains is NOT correctly matched with its function or purpose?

a) Gram-stain: A staining technique that stains gram-positive and gram-negative bacterial cells different colors.
b) Simple stain: A staining technique that can stain all types of bacteria cells, including bacterial endospores.
c) Acid-fast stain: A technique used to stain bacteria that possess mycolic acid in their cell walls.
d) Capsule stain: A staining technique that stains the background behind bacteria to allow their capsules to visually stand out.

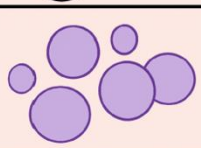
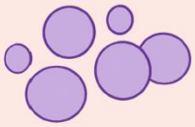
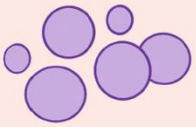
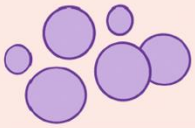
PRACTICE: Which of these is considered a differential stain and why?

a) Flagella stain: differentiates the flagella from the rest of the structures of the cell.
b) Capsule stain: differentiates the capsule from the background behind the bacterium.
c) Gram-stain: differentiates two types of bacteria, gram-positive and gram-negative.

CONCEPT: GRAM STAIN

●Recall: _____**-Stain**: a *differential* stain separating bacteria into 2 major groups based on *cell wall* differences.

□ Consists of a series of _____ steps:

Gram-Staining		Gram ⊕	Gram ⊖
Steps	**State of Bacteria**		
1 Sample is stained with *crystal* _____ dye.	All cells are stained **purple**.		
2 Sample is treated with an _____ solution.	All cells remain **purple**. Iodine ensures crystal violet dye is affixed to gram ⊕ cells.		
3 Sample is treated with _____ (alcohol & acetone).	Gram ◯ cells remain **purple**. Gram ◯ cells become colorless.		
4 Sample is stained with the counterstain _____.	Gram ◯ cells remain **purple**. Gram ◯ cells become **pink**.		

PRACTICE: Which of the following answers lists the steps of gram-staining in the correct order?

a) Stain with primary stain (crystal violet), add iodine, add decolorizer, stain with counterstain (safranin).
b) Add iodine, add decolorizer, stain with primary stain (crystal violet), stain with counterstain (safranin).
c) Stain with primary stain (crystal violet), add decolorizer, stain with counterstain (safranin), add iodine.
d) None of the above are correct.

PRACTICE: Which of the following reagents is used to stain gram negative cells pink (or red) in the gram stain?

a) Iodine.
b) Safranin.
c) Crystal violet.
d) Decolorizing agent.

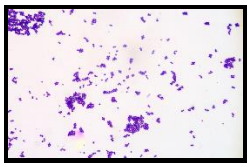
CONCEPT: GRAM STAIN

PRACTICE: Which of the following statements is false?

a) Crystal violet is used to stain the cells purple.
b) Alcohol is used to kill the bacteria that are not stained.
c) Gram positive cells end up being stained purple.
d) Iodine is used to affix the crystal violet dye to gram positive cells.

PRACTICE: Results from a gram stain show that these bacteria are:

a) Gram negative.
b) Gram positive.
c) Gram neutral.



PRACTICE: A scientists has a sample with two different species of bacteria. The first species is *Staphylococcus aureus*, a gram-positive bacterium. The second species is *Escherichia coli*, a gram-negative bacterium. The scientist gram-stains his sample of bacteria. What colors will the two species of bacteria be after staining?

a) *Staphylococcus aureus*: pink; *Escherichia coli*: purple.
b) *Staphylococcus aureus*: purple; *Escherichia coli*: pink.
c) Both *Staphylococcus aureus* and *Escherichia coli* will be purple.