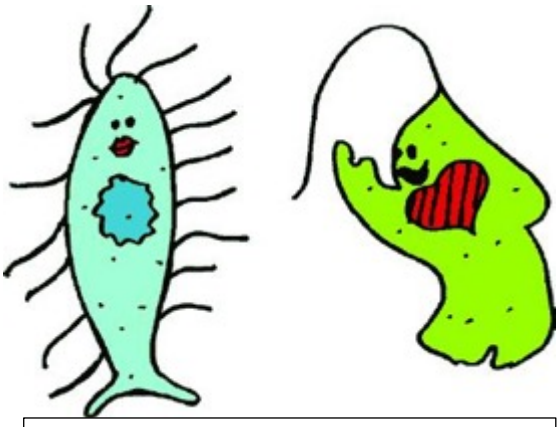
Lecture 1

I. _____ is the study of _____ _____. Special techniques are required to isolate, grow, and/or visualize these agents and microorganisms.

II. Ubiquity and Importance of Microorganisms

A. Where are microorganism found

1. _____
2. Food
3. Water
4. _____
5. Clothing
6. **Bed sheets**
7. Air
8. _____
9. Acid
10. _____
11. Feces



Remember: Pure cultures are very uncommon in nature. **When working with sterile media and pure cultures, it is important to use sterile technique.**

B. Where aren't they?

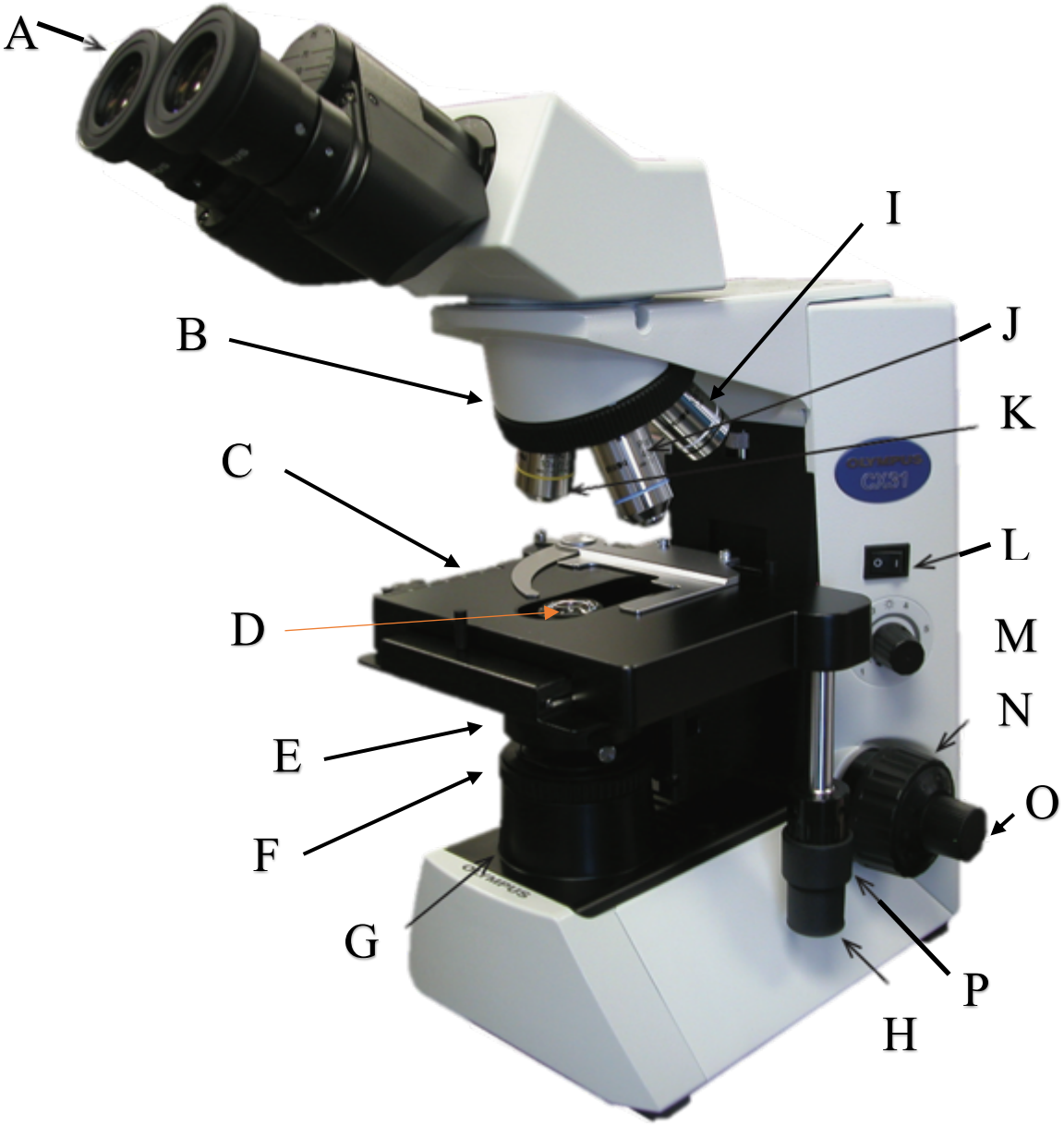
1. _____
2. In the interior of a healthy human body, excluding the digestive tract (e.g. _____, cerebral spinal fluid, _____, bone marrow, urine while it's in the bladder).

C. Without microbes:

1._____ and dead plants and animals would not even decompose.
2. Without photosynthetic microorganisms, _____.
3. _____.

D. Despite the ubiquity of microorganisms, they_____. Sterile media stays sterile until inoculated and then, if inoculated with a single microorganism, the culture is a _____.

Parts of Microscopes



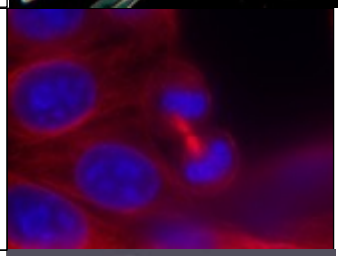
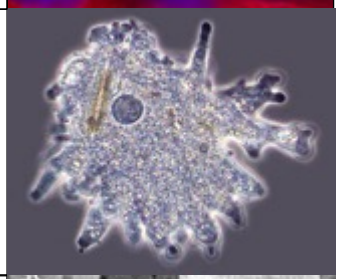
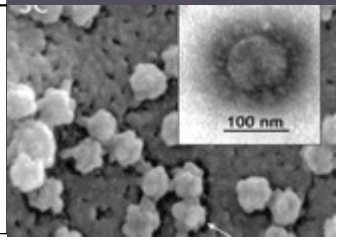
A	_____ Magnify 10X	I	Oil-immersion objective lens: Magnifies _____
B	Nosepiece	J	High-dry objective lens: Magnifies _____
C	_____: Holds specimen	K	Low-power objective lens: Magnifies _____
D	Condenser: _____onto the specimen.	L	Power switch
E	_____: Controls the amount of light that enters the objective.	M	Knob to control the _____of light
F	Field iris diaphragm: Controls the amount of light leaving the source	N	Coarse adjustment focusing knob: Makes _____ _____ in focus.
G	Light source	O	Fine adjustment focusing knob: Makes _____ _____ in focus.
H	X-axis control knob: Moves specimen _____ in the field of view	P	Y-axis control knob: Moves specimen _____ in the field of view

III. Microscopy

A. Types of microscopes:

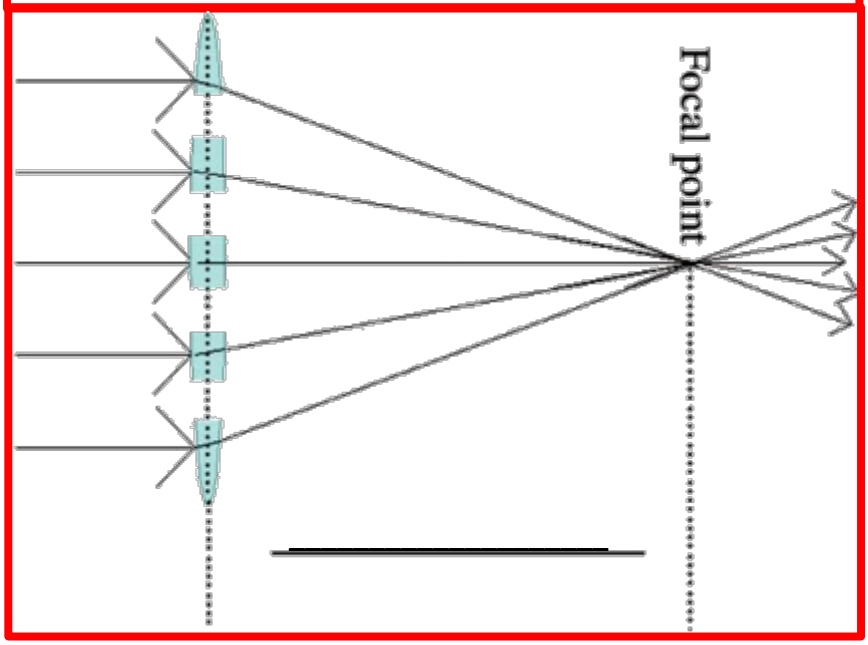
1. _____
   a. Forms _____.
2. The _____
   a. In this type of microscope, a hollow cone of light is focused on the specimen in such a way that only light reflected or refracted by the specimen forms an image. The image appears as _____ _____
   b. Allows for visualization of considerable _____ in larger eukaryotic microorganisms. Also, it is better for visualizing _____.
3. The phase-contrast microscope
   a. Converts slight differences into refractive index and cell density into _____.
   b. Often used to observe _____.
4. _____
   a. Exposes a specimen to UV, violet, or blue light and forms an image of the object with the resulting fluorescent light.
5. _____
   a. _____is focused on the specimen using magnetic lenses in a vacuum. The _____ passing through it and the beam is focused by magnetic lenses to form an enlarged visible image of the specimen on a fluorescent screen.
   b. The resolution is _____.
   c. Capable of well over _____.

Brief Recap: Types of Microscopes

Dark Field Microscope A hollow cone of light hits the specimen and only refracted and reflected rays enter the objective. Image is bright against a dark background. Shows internal structures. For image, go to http://microbewiki.kenyon.edu/index.php/File:Diatoms_in_dark_field_lighting.jpg.	
Fluorescence Microscope Short wavelength (UV, Blue) light is focused on the specimen and fluorescence emission forms the image. Can be used to view cells attached to an opaque surface. Photo by Amy Rhoad	
Phase Contrast Microscope Changes small differences in refractive index and cell density into large variations in light intensity (enhances contrast). For an image, go to http://www.microscopy-uk.org.uk/intro/illu/phase.html	
Electron Microscope A beam of electrons hits specimen and is scattered. Image forms on a fluorescent screen. For an image, go to https://phil.cdc.gov/Details.aspx?pid=6400	

How Does It Work?
Light Microscopes

A light microscope is a collection of mirrors and lenses.
Microscope lenses act like a _____.



C. Magnification and Resolution

1. Magnification

a. The process of _____ as an **optical image**.

b. The shorter the _____, the greater the _____. (See How Does It Work? Focal points, right)

c. The total magnification of a microscope is the _____ of the magnifying power of the _____ and the _____ lenses.

Important formula to remember:

Objective lens x Ocular lens = Total magnification

For example:
Low power: (10X)(10X) = 100X
High dry power: (40X)(10X) = 400X
Oil immersion power: (100X)(10X) = 1000X

For example: What is the total magnification of our light microscopes if the 40X objective lens is being used?

_____

2. Resolution

a. The _____ objects that are _____. The better the resolving power, the closer the objects can be and still be seen as _____.

b. Resolution is _____when _____ wavelengths of light are used.

c. Lenses with _____ have shorter _____ and better resolution. (See How Does It Work? Resolution, above)

d. Immersion oil

i. Oil has the _____

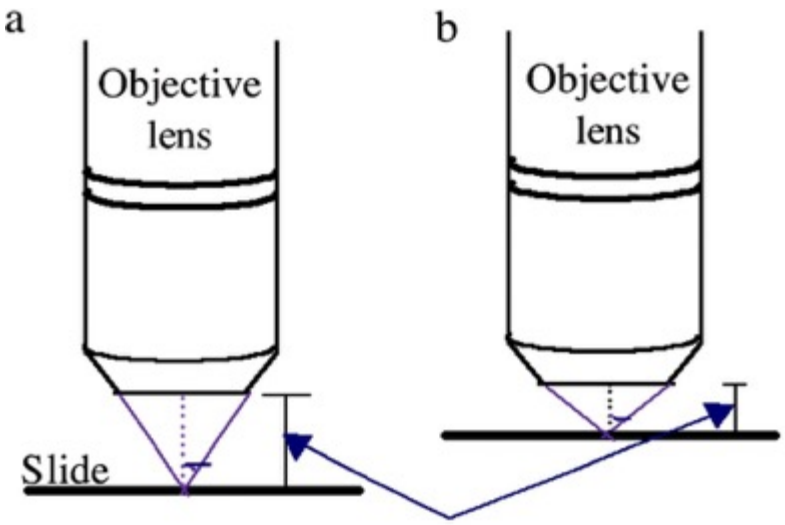
ii. Rays that would not enter the objective in air, due to reflection and refraction, can do so in oil. This effectively _____.

e. Parfocal

i. In a microscope that is parfocal, the image should _____ when the objective lens is changed.

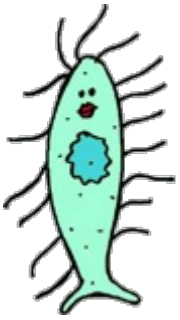
How Does It Work?
Resolution

Working distance: The distance between the front surface of the lens and the surface of the cover glass or specimen when it is in focus.

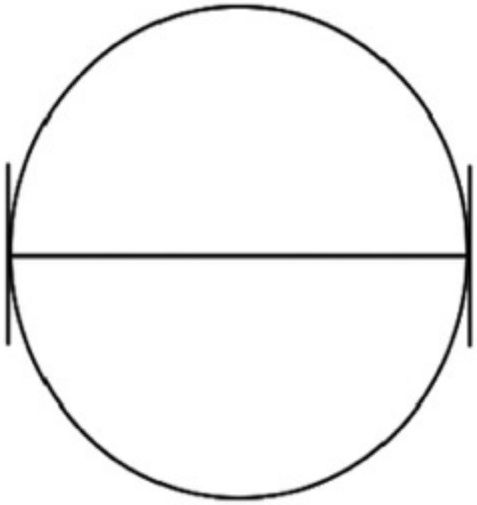


If the same wavelength of light is being used, which lens (a or b) has the greater resolution?

_____



The diameter of the field of view at 100X total magnification for our microscopes has already been measured.



f. Determining the _____ of objects

i. Size can be estimated by first calculating the _____ of the field of view at 100X total magnification. This can be done by viewing a ruler etched on a glass slide.

ii. Now, by estimating _____ by a microorganism, size can be estimated.

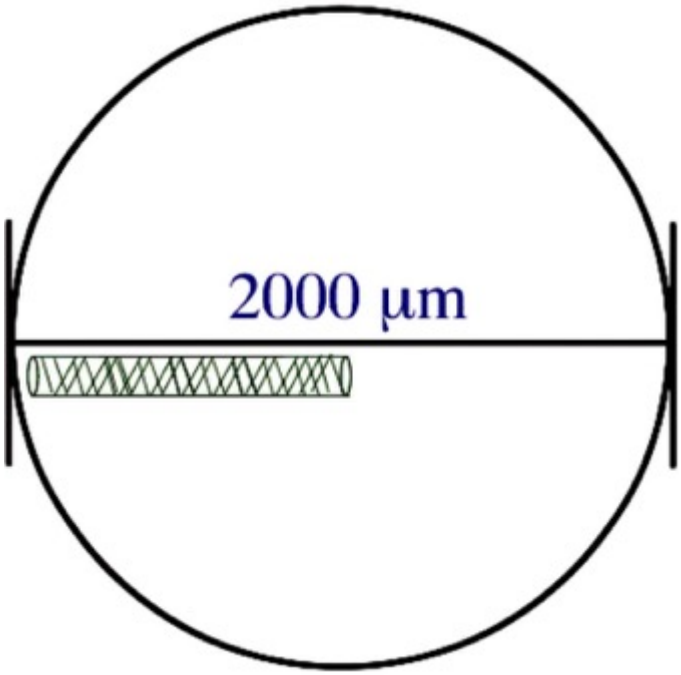
iii. There is an _____ relationship between the _____ and the _____. The greater the magnification, the closer the object appears and the smaller the size of the field.

	Total Magnification		
Field diameter	100X	400X	1000X
	2000 µm	2000 µm/4 = 500 µm	2000 µm/ 10 = 200 µm

Test your understanding:

1. Using what you just learned about determining the size of a microorganism, approximately how long is this specimen?

_____



2. How much of the field of view would the microorganism above take up if the total magnification was 400X?

_____