MCB 2010L

DR LARRYN FARRIS

Welcome!!!!!!

About Me

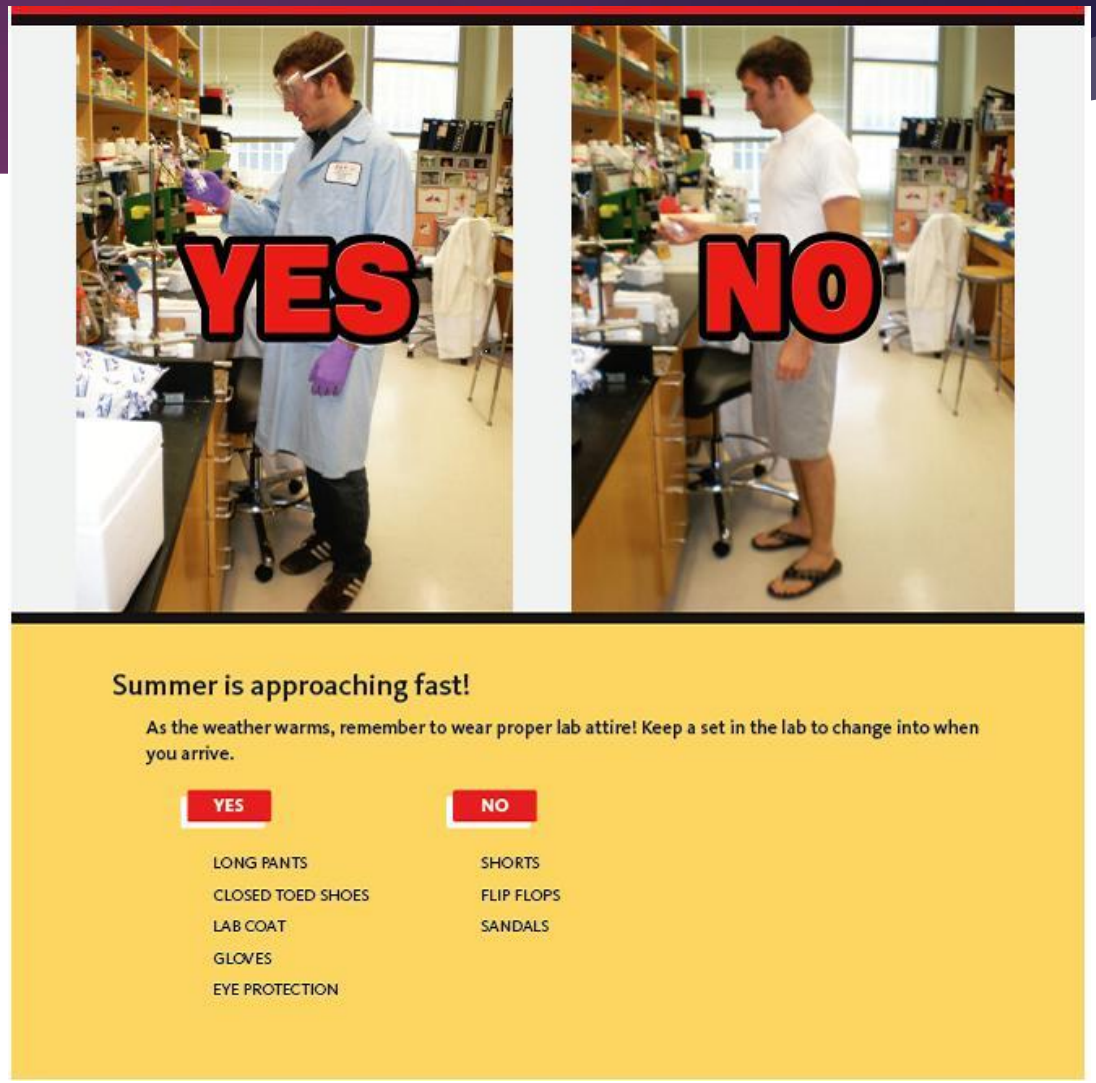
- Bachelor of Science in Molecular Biology and Microbiology from University of Central Florida in 2012
- Master of Science in Biology with area concentration in Molecular Genetics and Biochemistry from Georgia State University in 2014

More About Me

- Doctor of Pharmacy and Master of Public Health degrees from Nova Southeastern University in 2020

Requirements for Lab

- Lab Coat
- Safety goggles
- Closed-toed shoes
- Lab Manual
- Behave safely at all times
- **Attendance**



How will lab operate?

- We will meet EVERY week at 5:45 pm promptly
  - Office hours will be held from 4:45 pm until 5:45 pm
- Lectures will be pre-recorded and posted on Blackboard
  - Watch the lecture prior to class
- There will be weekly assignments (either a quiz or discussion post)
  - 5 quizzes and 5 discussion posts (alternate weeks)
  - Open on Tuesdays at 12:00am and close on Sundays at 11:59pm on the week due
  - No make ups or late submissions
- Youtube videos are posted on Blackboard – these correspond to weekly assignments
  - I recommend viewing the videos prior to class

Microscopy

The Microscope

- Coordinated system of lenses arranged to prodice an enlarged, focusable image of a specimen
- Magnification – increases apparent size
- Resolution – ability to distinguish two points as separate
- Contrast – the amount of difference between the lightest and darkest parts of an image (artificial dyes increase contrast and make the specimen more visible)
- Invention formulated the cell theory and the study biological structure at the cellular level.
- Fundamental tool in biology

The Compound Microscope

- Microscopes are optical instruments that permit us to view the microbial world
- Lenses produce the magnified images that allow us to visualize the form and structure of these tiniest of living beings

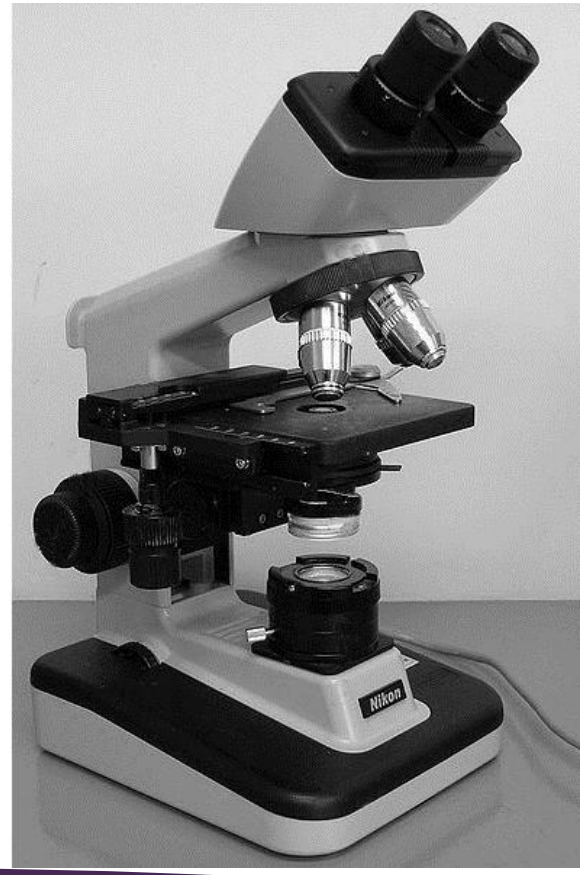
Parts of the Compound Light Microscope

- Illuminating system
  - Light source – lightbulb
  - Condenser lens – focuses light onto the specimen
  - Condenser iris diaphragm – regulated the amount of light reaching the specimen
- Imaging system
  - Improves resolution and magnifies the image
  - Objective lens (four different magnifications)
  - Ocular lens – the lens you look through
  - Stage – secures the slide

Ocular lenses	_____
Objective lenses	_____
(Revolving) Nosepiece	_____
Stage and stage clips	_____
Course and fine focus knobs	_____
Condenser lens	_____
Iris diaphragm	_____

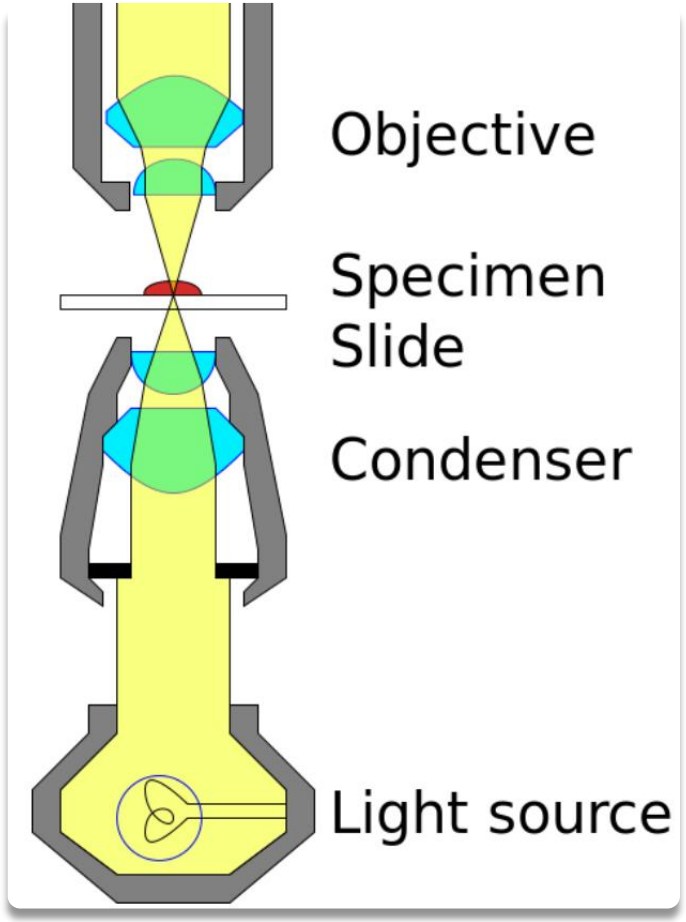
Locate the parts on the microscope that allow you to:

- Move the stage (stage adjustment knobs)
- Adjust the condenser lens
- Adjust the light intensity
- Adjust the iris diaphragm
- Adjust the distance between the ocular lenses



Exercise 1

The Path of Light



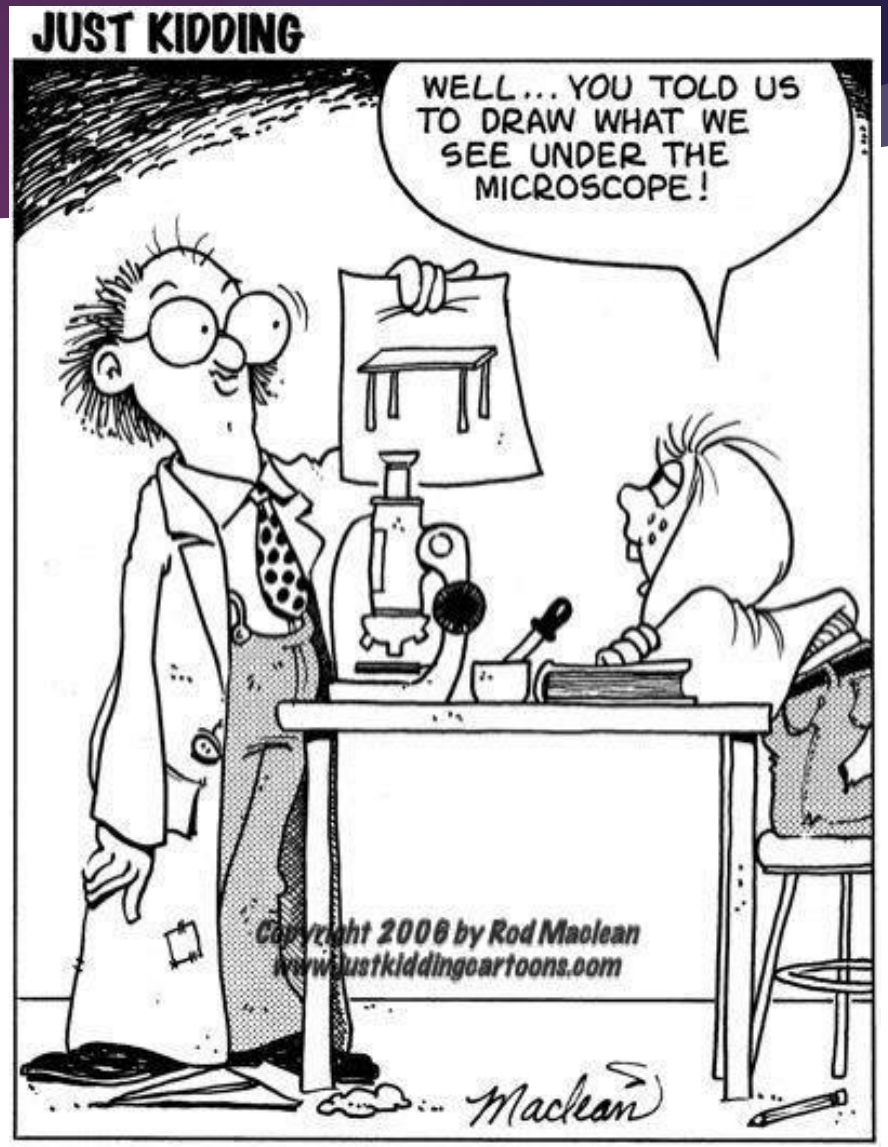
- Light waves that pass through and interact with the object may speed up, slow down, or change direction as they travel through "media" (such as air, water, oil, cytoplasm, etc.) of different densities
- For a compound microscope, the optical path leading to a detectable image involves two lenses – the objective lens and the ocular lens
- The objective lens magnifies the object and creates a **real image**, which will appear to be 4, 10, 40, or 100 times larger than the object actually is, depending on the lens used
- The ocular lens further magnifies the real image by an additional factor of 10, to produce a vastly larger **virtual image** of the object when viewed by you

Magnification

- The microscope you'll be using in lab has a compound system of lenses
- The objective lens magnifies the object "X" number of times to create the real image, which is then magnified by the ocular lens an additional 10X in the virtual image
- Total magnification, or how much bigger the object will actually appear to you when you view it, can be determined by **multiplying the magnification of the objective lens by 10**
- **The ocular lens magnification is ALWAYS 10**

Total magnification

- $Mag_{Tot} = Mag_{Obj} \times Mag_{Ocu}$
- Mag_{Tot} is the total magnification of the image
- Mag_{Obj} is the magnification of the objective lens
- Mag_{Ocu} is the magnification of the ocular lens



	Magnification of objective lens	Total magnification of viewed object
Scanning Lens		
Low Power Lens		
High Power Lens		
Oil Immersion Lens		

Exercise 2

Resolution

- Resolution is the minimum distance between objects needed to be able to see them as two separate entities
- It can also be thought of as the size of the smallest object that we can clearly see
- The practical limit of resolution for most microscopes is about 0.2 μm
- Cells of all types of organisms lack contrast because many cellular components refract light to a similar extent (especially true of bacteria)
- To overcome this problem and increase contrast, biological specimens may be stained with selective dyes

Field of View

- The area you can see through the ocular and objective
- Can be used to determine the approximate size of an object
- Measured in micrometers
- Ocular micrometer is a small glass disk with thin lines numbered and etched in a row
- Must calibrate the ocular micrometer by comparing its lines to those on a standard ruler (stage micrometer)
- Stage micrometer is a glass slide having precise spaced lines etched at known intervals

Oil Immersion Objective Lens

- Lens with highest magnifying power
- Achieves a total magnification of 1000X with a resolution of 0.2 μm
- The resolving power of this lens is dependent on "immersing" it in a drop of oil
- Prevents the loss of at least some of the image-forming light waves because of refraction
- It is important to remember that **you must use a drop of oil** whenever you use the **oil immersion objective** or you will not achieve maximum resolution with that lens
- **You should never use oil with any of the other objectives**

Using the Microscope

- Begin with the 4x objective lens, find your specimen and put it in the center
- Once in the center, increase the magnification to 10x
- You should not have to move the specimen (it will be in the center) the only adjustment is to see the specimen clearly (resolution)
- Once you can see it at 10x, increase the magnification to 40x
- Again, You should not have to move the specimen (it will be in the center) the only adjustment is to see the specimen clearly (resolution)
- Once you see the specimen at 40x, move the stage down so that you can add your drop of oil
- **Most important fact** about oil immersion lens, once the oil is placed do NOT move the specimen!! Only adjust the resolution using the fine adjustment knob

Questions??

Thank you!!! Stay Safe